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Ecosystem level impacts of annual and perennial
N₂-fixing invasive alien plants in the fynbos
vegetation of South Africa

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Abstract

In the nutrient-poor fynbos, changes in soil nutrients can affect species composition and community structure. The N₂-fixing ability of invasive alien plants, therefore, may have impacts on ecosystem and community functioning within the fynbos vegetation type. This study investigated changes in fynbos N cycling regimes with the invasion of two different N₂-fixing invasive alien plants, *Acacia saligna* and *Lupinus luteus*. Specifically, the following questions were asked: (1) Do N₂-fixing invasive alien plants increase rates of nitrogen cycling through high N inputs in fynbos? (2) Are there differential effects between the two N₂-fixing invasive alien species on the same soil type? (3) Will the growth of weedy grass species be enhanced by higher levels of N in soils from stands of N₂-fixing invasive aliens? (4) Will the clearing of acacia stands increase N mineralization rates in soils due to changes in soil microclimate? Plots were established in uninvaded fynbos, and in invaded grassland, lupin, and acacia areas to compare perennial N₂-fixers and non-fixers (acacia vs. fynbos) as well as annual N₂-fixers and non-fixers (lupin vs. grass). Plots were also established in cleared acacia stands to look at the possible impacts of ongoing clearing efforts. In all of these treatments, data were obtained on litterfall, litter quality, static soil nutrient pools, net N mineralization, and litter decomposition. In addition, a bioassay experiment was used to ascertain whether a weedy grass species would be more likely to invade after acacia invasions. Both N₂-fixing invasive alien species were able to switch and maintain N cycling regimes within the otherwise low nutrient soil of the fynbos. Acacia and lupin, when compared to their respective non-fixing communities, had greater amounts of litter with higher nitrogen concentrations. The larger quantities of N being returned to the soil from the aboveground biomass resulted in higher levels of soil organic matter and total N in acacia stands, but not in lupin stands. This difference in static soil N pools was due to higher rates

of decomposition in lupin litter than any other litter type in the study. Both N₂-fixing invasive alien species, however, increased rates of net NH₄⁺ and NO₃⁻ mineralization, thereby increasing rates of production of available inorganic N. It is predicted that the perennial nature of acacias will result in annual incremental increases in soil N pools and N mineralization rates. The annual lupin system, however, will achieve a steady state of N cycling where N mineralization rates remain constant from year to year. Comparisons with other studies of N₂-fixing invasive alien plants suggest that any N₂-fixer may affect N cycling regimes if the invaded system has low pre-invasion rates of N cycling and enough N rich litter is added by the invasive species through time. The alteration of N availability by acacias was shown to increase growth rates of the weedy grass *Ehrharta calycina*. This data suggests that secondary invasions by nitrophilous weedy species may occur after clearing N₂-fixing alien species in the fynbos, continuing the high N cycling regime and making recolonization by native species difficult. In treatments cleared of acacias, there was an increase in soil temperature but no change in net N mineralization rates. The current alien plant clearing initiatives in South Africa, therefore, will not result in N mineralization flushes over and above the rates that already exist. It is suggested that although fire will decrease N pools and N mineralization rates through N volatilisation, acacia systems will start the next successional sequence with higher N cycling rates than uninvaded systems. Frequent fires through acacia stands will result in less apparent changes in N cycling regimes because of the constant combustion of organic N pools. Finally, implications of the data for restoration of native fynbos vegetation after clearing invasions of N₂-fixing invasive alien plants are discussed. It is suggested that managers add a C rich mulch to soils after burning to help immobilise high levels of available N.

1 Introduction

The identity of a plant species can have distinct effects on soil nutrient cycling (Vitousek and Walker 1989, Wedin and Tilman 1990, Vinton and Burke 1995, Wardle et al. 1998, Garcia-Montiel Binkley 1998). Changes in nutrient cycling can then feedback to affect plant growth and litter quality, creating a continuous loop or cycle (Vitousek 1982, Hobbie 1992). When plant species change in a community, the ecosystem properties can shift into a new regime that is self-maintained through these feedback cycles (Vitousek 1982, Hobbie 1992, Vinton and Burke 1995). This process has been clearly demonstrated with invasions of nitrogen (N hereafter) fixing invasive alien plant species in Hawaii, California, and South Africa which have led to shifts from low to high N cycling regimes (Vitousek et al. 1987, Vitousek and Walker 1989, Stock *et al.* 1995, Maron and Connors 1996, Pickart *et al.* 1998a, Maron and Jefferies 1999). Constant inputs of N rich litter through time by invading species have increased pools of organic soil N. Because the invading species can fix their own N, they are not limited by soil available inorganic N. Lower nutrient use efficiencies due to a lack of N limitation result in litter that has lower C:N ratios than native vegetation. The larger pools of organic matter in the soil, therefore, also have lower C:N ratios than in uninvaded vegetation. Decomposition and net N mineralization rates are higher in soils under invasions because microbial communities do not need to immobilize N in the high quality litter. Because net N mineralization is the transformation of organic to inorganic N (NH_4^+ and NO_3^-), as rates of mineralization increase so do the levels of plant available inorganic N in soils. In California, this type of ecosystem shift to a system with high production rates of plant available N has led to secondary invasions by nitrophilous weedy species (Maron and Connors 1996) completing the feedback cycle.

Changes in N cycling regimes may have the largest effects on systems that are especially low in nutrients (Vitousek and Walker 1989). The Cape Floristic Region of South Africa (also known as fynbos) is the most nutrient poor (Read and Mitchell 1983) and species rich of all of its Mediterranean counterparts (Cowling *et al.* 1992). Within this ecosystem, edaphic characteristics of soils, including N, can determine community structure and species presence or absence (Richards *et al.* 1997a,b). Speciation patterns within the fynbos may also be driven by soil properties (Cowling *et al.* 1992). In addition, some native fynbos species have shown decreases in growth rates with increased levels of N or P (Lamb and Klausner 1988, Witkowski 1989a). Because of these characteristics, shifts to high N cycling in soils may have large impacts on community structure and function.

Nitrogen cycling in the natural vegetation of the fynbos is a slow process (Stock and Allsopp 1992). The vegetation consists largely of sclerophyllous shrubs that grow slowly and have high rates of internal nutrient recycling prior to leaf abscission. The small quantities of litter that fall have high levels of lignins and soluble phenolic compounds and low levels of tissue nitrogen (Mitchell *et al.* 1986). During the decomposition of leaves, nitrogen must be immobilized into the litter before mineralization can take place (Mitchell *et al.* 1986, Witkowski 1991). Because leaf decomposition takes so long, it is estimated that the major route of nutrient release in the fynbos is through the fires that naturally occur at approximately 20 y intervals (Stock and Lewis 1986, Mitchell *et al.* 1986).

Although native N₂-fixers exist in the fynbos, they are short lived and distributed patchily in the post fire community. These species do not deposit enough of their N rich litter to make an impact on soil N status (Cocks and Stock 2000). Invasive alien N₂-fixing *Acacia* species, on the other hand, can form dense stands that regenerate after each fire cycle (Milton 1981). These stands of alien trees are taller than the native fynbos (van Wilgen and Richardson 1985) and contain larger quantities of biomass (Milton 1981). The resulting

steady state communities of invasives (Richardson and Cowling 1992) have constant, large inputs of N rich litter. For *Acacia cyclops* and *A. saligna*, greater amounts of litter with higher leaf N concentrations resulted in higher levels of total soil N and organic matter (Witkowski 1991). Changes in soil N pools led to higher rates of net N mineralization in *A. cyclops*, although not *A. saligna* stands (Stock *et al.* 1995).

The lack of increased N mineralization in *A. saligna* stands may have been due to species or soil differences, but which of them was the causal factor could not be determined. The sampled *A. saligna* and *A. cyclops* stands were found on acid sandplain lowland fynbos and strandveld soils, respectively. Under native vegetation, strandveld soil is less acidic and has larger pools of total N and P (see Stock *et al.* 1995 for detailed descriptions). Species differences (i.e., *A. saligna* vs. *A. cyclops*) may have been responsible for the differential effects on N mineralization through litter quality differences (e.g., Wedin and Tilman 1990, Vinton and Burke 1995, Wardle *et al.* 1998, Christian and Wilson 1999). The soil differences (i.e., fynbos vs. strandveld soils) may have been responsible for the differential effects on N mineralization rates through differences in microbial communities and abiotic factors (e.g., Alexander 1977).

Research surrounding changes in N cycling in relation to the restoration of fynbos vegetation is becoming highly relevant due to new environmental legislation regarding invasive plants passed by the government. The Working for Water programme, established in South Africa in 1995, was spearheaded to remove water-demanding invasive alien trees from waterways. This globally unparalleled initiative is expected to save money in the long term by avoiding the need for future dams. At the same time, the Working for Water programme is creating over 35,000 jobs for previously unemployed people (van Wilgen *et al.* 1998). The equivalent of 1.7 million ha, containing about 15 invasive woody species, is estimated to be cleared by the year 2015 (van Wilgen *et al.* 1998). Although the *Acacia* spp.

are only a part of this pool, large areas of land invaded by N₂-fixing invasive aliens are to be cleared, forecasting possible problems due to high nitrogen soils for native species trying to recolonize.

Just as in the coastal dunes of California (Maron and Connors 1996), increases in rates of N cycling may lead to secondary invasions by weedy nitrophilous species (Davis *et al.*, 2000) once acacia stands are cleared by the Working for Water programme. Fynbos plants are pre-adapted to low nutrient conditions, having slow growth rates that are not plastic in relation to soil nutrient levels (Stock and Lewis 1984, Witkowski *et al.* 1990b). As mentioned earlier, native species can actually be negatively affected by increases in P and N (Lamb and Klaussner 1988, Witkowski 1989a). The invasive alien grass species that exist in the fynbos, however, are ruderal species, which increase growth rates in response to fertilization (Simons 1999).

These physiological differences in combination with specific life history patterns will be beneficial to weedy grass species after alien clearing. The successional nature of the fynbos is such that most plant species germinate immediately after fire and few species colonize in the interval between fires (Bond and van Wilgen, 1996). This creates a system in which plant interactions in the year following a fire determine community structure. Although competition for resources is not an accepted model of plant interactions in the fynbos (Bond *et al.* 1992), high levels of soil nitrogen may enhance growth of invasive grass species, creating a system in which competition exists. The ruderal species, which can quickly use excess nutrients to add biomass, may quickly overtop native seedlings. Native species might then drop out of the community due to shade intolerance (Cowling and Gxaba 1991). The lack of native seed banks after invasions of acacias (Holmes and Cowling 1997b) in conjunction with the slow dispersal rates of native species will further hinder recolonization of fynbos plants after acacias are cleared (Holmes and Richardson 1999).

Indeed, increases in alien grass abundance in the fynbos have already been noted after the clearing of long term invasions of *A. saligna* (Holmes and Cowling 1997b), in areas of high disturbance (Jobst 1996), and may also be increasing due to atmospheric N deposition (Wilson 1999). Within Riverlands Nature Reserve, land cleared of *A. saligna* 1-2 years ago has high covers of the grass *Ehrharta calycina* (personal observation). Although native, this species is not dominant at any time in the post fire succession of acid sand lowland fynbos (Hoffman 1984, Hoffman *et al.* 1987), and is generally a colonizer of highly disturbed areas. Weedy grass species such as these will not only exert competitive pressures on native species, but will also continue to add N rich litter to the soil and may therefore perpetuate high rates of N cycling (Vinton and Burke 1995) even after acacias are cleared.

Another possible effect of clearing large areas of acacia stands is an increase in N mineralization due to elevated soil temperatures after the tree canopy is removed. Increases in soil temperatures as small as 5 –10°C can stimulate microbial communities resulting in greater quantities of available N (Nadelhoffer *et al.* 1991, Verburg *et al.* 1999). This mechanism of enhanced mineralization due to the removal of aboveground biomass and elevated temperatures, has been used to explain flushes in NH_4^+ and NO_3^- after fires in grasslands (Blair 1997). Increased N mineralization after acacia clearing may exacerbate the detrimental effects of high N cycling for native vegetation recolonization.

In order to explore the concept of ecosystem shifts and, at the same time, aid managers in efforts to restore degraded land back to indigenous vegetation, this project investigated changes in nitrogen cycling with two N_2 -fixing alien species in Riverlands Nature Reserve, Western Cape, South Africa. Riverlands, located 63 km north of Cape Town, consists of acid sandplain lowland fynbos in a mosaic of land use history (see Section 2.1). The two species investigated were *A. saligna* (Labill.) Wendl. and *Lupinus luteus* L. (hereafter acacia and lupin, respectively). The research was aimed at answering the

following questions: (1) Do N₂-fixing invasive alien plants increase rates of nitrogen cycling through high N inputs in acid sand lowland fynbos? (2) Do the two species of N₂-fixing invasive alien species on the same soil type lead to differential effects on N cycling? (3) Will the growth of weedy grass species be enhanced by higher levels of N in soils from stands of N₂-fixing invasive aliens? (4) Will the clearing of acacia stands further increase net N mineralization rates in soils due to altered soil microclimates?

Each invasive species was studied in conjunction with its respective community: acacias were compared to uninvaded fynbos and lupins were compared to grassy areas within old fields as well as uninvaded fynbos. Nitrogen inputs into soils were quantified through measures of litterfall and leaf N concentrations, as well as the static pools of soil organic matter, total N, total P, and various cations. The rate at which N inputs would become available to plant communities was quantified by measuring net NH₄⁺ and NO₃⁻ mineralization within field soils and decomposition rates of the litter in laboratory experiments. Finally, the probability of grass invasions was investigated in a bioassay experiment in which the native weedy grass *Ehrharta calycina* Smith was grown in soils from *A. saligna* invaded and uninvaded acid sandplain lowland fynbos areas.

2 Materials and Methods

2.1 Study Site:

The fieldwork was done on Riverlands Nature Reserve (33°32'S 18°35'E), 63 km north of Cape Town, South Africa (Fig 2.1.1). The native vegetation is broadly classified as acid sandplain lowland fynbos (Moll *et al.* 1984) or as the BHU (Broad Habitat Unit) Hopefield sandplain fynbos (Cowling and Hejnis 2000), some of which is located on seasonal wetlands. The vegetation is similar to that within the nearby Pella site (CSIR fynbos biome intensive study site) as described by Hoffman *et al.* (1987). The climate is Mediterranean, with hot dry summers and cool wet winters; annual rainfall for the Pella site averages 522 mm and the mean annual temperature is 17.3°C (Jarman and Mustart 1988). Riverlands soils are similar to those of Pella, consisting of well drained aeolian acidic sands approximately 1 – 2 m deep in the Constantia and Clovelly forms (Lambrechts and Fry 1988) according to the South African binomial classification (MacVicar *et al.* 1977). Riverlands, which is 1300 ha in size, hosts an estimated 400 plant species, 41 of which are critically rare and endangered (Kilian 1995). Riverlands consists of a farm that was purchased from Transnet (formerly South African Railways) in 1985 by Cape Nature Conservation (CNC) due to its high concentration of rare plants. Owing to its history, it consists of a patchwork landscape including invasive alien vegetation, fallow fields that were originally strip-plowed, and uninvaded fynbos.

In 1995, 300 ha were covered in dense alien vegetation, mostly *Acacia saligna* (Port Jackson willow), an invasive from Australia (Kilian 1995). By examining aerial photographs from 1960, 1968, 1977, and 1988, it was estimated that acacias established before 1968, dispersing into undisturbed fynbos from trees planted on the neighboring property. Due to the gall-forming rust fungus (*Uromycladium tepperianum*), which has been employed as a

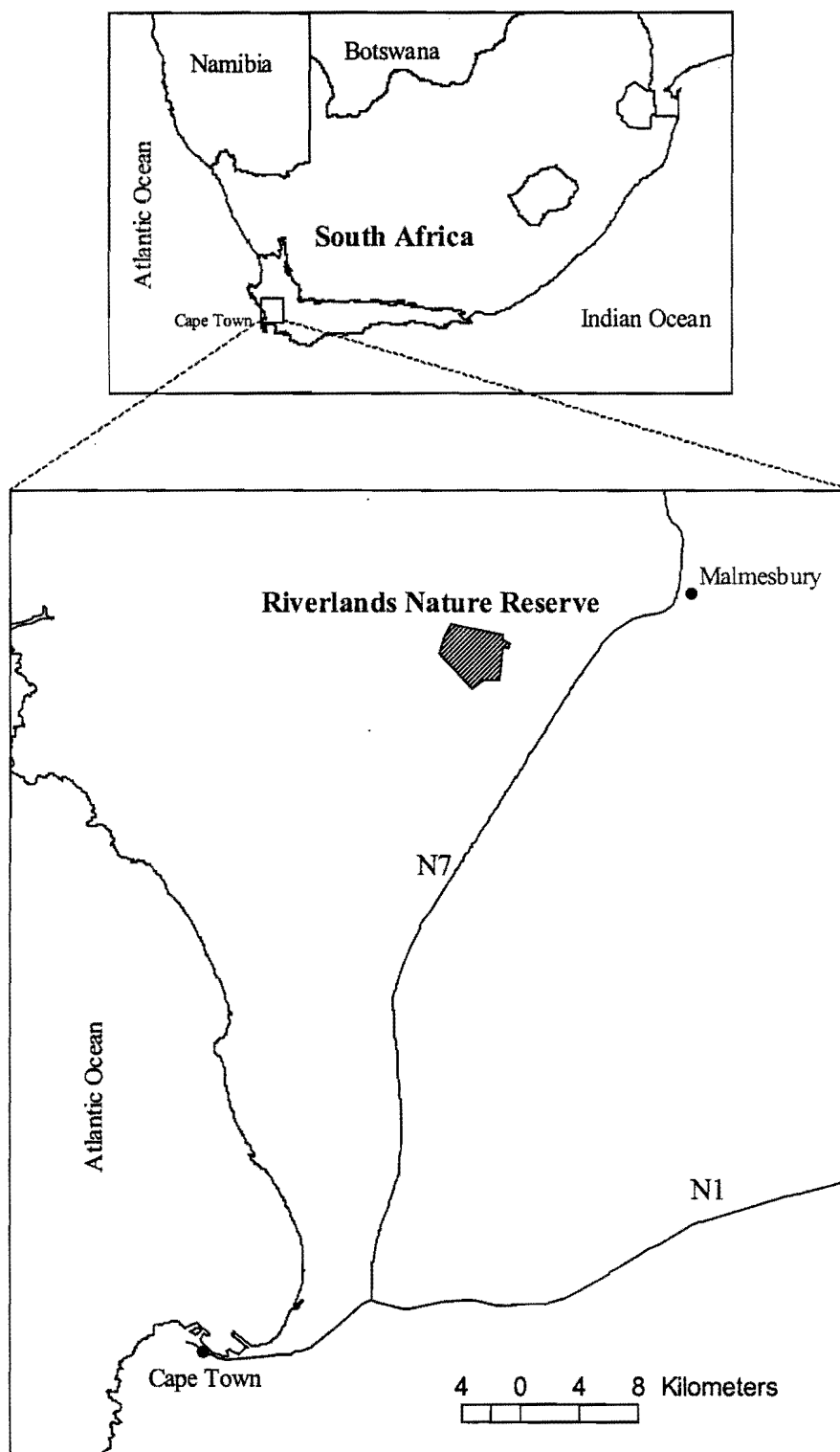


Figure 2.1.1. Map showing the Cape Floristic Region (shaded section in top map) in relation to southern Africa. The inset shows Riverlands Nature Reserve in relation to the cities of Cape Town and Malmesbury, South Africa, as well as the national highways N1 and N7.

biocontrol agent, densities of live *A. saligna* have decreased by as much as 80% since 1988 (Morris 1997). However, because dead trees tend to be kept upright by the sheer densities within stands (personal observation) and seed banks under *A. saligna* stands average 11,920 seeds m⁻² (Milton and Hall 1981, Dean *et al.* 1986), the biocontrol agent is not sufficient to eradicate the weed. In 1998, The Reconstruction and Development Program (RDP) awarded Riverlands a grant to clear its alien vegetation as a part of a poverty relief and job creation program for the people of the Riverlands community – a small settlement which has existed on the southern boundary of the farm/reserve for over a century. Acacias are cleared with the use of chainsaws, axes, and the subsequent stumps are sprayed with the herbicide Roundup. Riverland's managers hope to keep the reserve free of fires until all of the alien trees have been cleared so that one controlled burn can be employed to try to regenerate native vegetation (de Vlam, personal communication). The RDP and CNC plan to have *A. saligna* eradicated by 2004.

The fallow fields were strip-plowed, probably planted with a grain crop and rotated with the legume, *Lupinus luteus*, which still stands. *L. luteus* is an agricultural annual lupin from the Mediterranean Basin. Those parts of the old fields not covered in lupin are covered in a mix of annual (*Avena fatua*, *Lolium perenne*) and perennial (*Cynodon dactylon*) grasses and annual forbs. Using aerial photographs, it was estimated that the lupin and grass plots were cultivated before 1968 and have been left fallow since the creation of the Riverlands Nature Reserve in 1985.

2.2 Experimental Design:

To compare the effects of two different N₂-fixing alien invasive species and include controls for each, five species “treatments” were employed: lupin in old fields, grass in old

fields, acacia thicket, cleared acacia thicket and pristine fynbos. Fynbos alone was not considered a satisfactory control for the lupin treatments due to the possibility that the old fields had been fertilized while under cultivation. To look at the impacts of clearing acacia stands on N mineralization, a cleared acacia treatment was also added. Each treatment had three different sites, and each site had 4 plots, thereby creating 12 total observations per treatment. This split-block design was used to test site (soil and microclimate) as well as treatment (plant species) differences. Plots were 3 x 3 m and, except for the fynbos treatment, randomly assigned.

In the lupin sites, lupin density ranged between 50 and 90% of the total ground cover, grass density ranged between 5 and 20% of the total ground cover, and bare soil ranged between 0 and 15% of the total ground cover. Grass sites contained no lupin vegetation, but consisted of a matrix of annual and perennial grasses and annual forbes and very little bare soil (between 0 and 5% of the total ground cover).

In the fynbos sites, the dominant reseeding proteoid species within each of the three sites was chosen as a target species, and plots were centered around randomly selected individuals of this species. The target species were: site (1) *Protea scolymocephala* L. Reichard aged approximately 4 – 5 years within a restio dominated community; site (2) *Leucospermum parile* (Salisb. ex Knight) aged approximately 4 – 5 years within a proteiod dominated community; and site (3) *L. parile* aged approximately 5 – 6 years in a proteiod dominated community. For each of the three acacia thicket sites, an adjacent 18 m by 4.5 m space was completely cleared using chainsaws and axes by the RDP; this created four consecutive cleared 4.5 x 4.5 m plots (3 x 3 m plots with a 0.75 m buffer all of the way around). Only leaf litter was left intact in the cleared acacia plots.

2.3 Biomass, Litterfall, and N Return to Soil:

To quantify standing N pools and N return to the soil in annual treatments, lupin and grass biomass were sampled in early December 1998, (the beginning of the summer), when annual plants were composed of dead, dry, standing material. Four quadrats, 0.25 m² and 0.5 m² for grass and lupin, respectively, were clipped per plot. For the grass treatments, dead and living biomass were separated, dried for at least 48 hrs at 80°C, and weighed. The lupin treatments were separated into live and dead non-lupin material, and vegetative and reproductive lupin parts, dried for at least 48 hrs at 80°C, and weighed. Seeds were excluded from weighing and nutrient analyses since these do not decompose. In most plots, the live vegetation was largely made up of the perennial grass *Cynodon dactylon*, while the dead material was made up of *C. dactylon*, various annual forbs, grasses, and herbs. In the lupin treatments, dead non-lupin material also consisted of fallen lupin leaf litter that had already started decomposing and, therefore, could not be separated out.

To measure litterfall in the perennial treatments, 4 (20 cm x 16 cm) mesh bottom litter traps were placed in each of the fynbos and acacia thicket plots in February, 1999. Litter was allowed to collect in the traps for 3 months at a time, after which the litter was collected, dried for at least 48 hrs at 80°C, separated and weighed. For acacia plots, litter was separated into leaves, pods, and flowers. For fynbos plots, litter was separated into leaves and flowers.

All litter was coarse ground with a 1 mm mesh to be used in decomposition microcosms (see below). Once the separate fractions were weighed, average proportions for each treatment were calculated and mixed such that 10 g of coarse ground material was achieved (Table 3.5.3). The fynbos treatment was averaged by site (rather than treatment) due to differences in stand age and species composition. Subsamples of the pre-mixed fractions were ground to a fine powder using a Retsch (Germany) mixer mill and analyzed for total N and total P. Total N was determined using a Kjeldahl digest with 0.1 g plant

material and subsequent colorimetric determination of ammonium (Stock and Lewis 1986). All C:N ratios were calculated assuming that leaf material was 45% C (Stock and Lewis 1986). Total P was determined using 0.1 g plant material as per the methods of Murphy and Riley (1962).

Samples of live *C. dactylon* from the grass and lupin plots were also dried and finely ground. Total N was analyzed to compare plant N uptake between N₂-fixing and non N₂-fixing treatments.

2.4 Net N Mineralization:

Net N mineralization was assessed for each of the five treatments using ion exchange resin bags (Gibson 1986, Richards *et al.* 1997b). Resin bags were created by adding 5 g of wet Amberlite resin (anion = IRA-400; cation = IR-120 plus) to nylon stockings cut to 5 cm². These were sealed with an electric glue gun. Bags were regenerated by being alternately rinsed with distilled water and shaken with 5% HCl for 30 minutes three times, and then a final three washes with distilled water. In the field, holes were dug at the center of the plot or beneath the target plant, and a spatula was used to lift the soil at the side of the hole to create a slot for the bags. In this way, one anion and one cation resin bag were placed next to each other beneath 5 cm of undisturbed soil. These were taken out and sealed into clean plastic bags after approximately 30 days, at which time newly regenerated resin bags were placed in the plot. Resin bags were alternated to give monthly quantifications of nitrogen mineralization for a full year.

Once taken out of the field, resin bags were kept in a freezer and eluted within 48 hours. The bags were cleaned by brushing off dirt, pulling off small roots, and rinsing briefly with distilled water. Each bag was then shaken separately with 50 ml of 5% HCl for 30

minutes. The eluates were stored in glass vials at 4°C until ammonium (NH_4^+) and nitrate (NO_3^-) analyses were performed. NH_4^+ (from cation resin bags) was determined using an indo-phenol blue method and NO_3^- (from anion resin bags) was determined with a copper-cadmium reduction followed by a nitrite analysis by the Griess-Ilosvay method (Stock 1983, Appendix 1).

Absorption efficiencies were calculated for both anion and cation resin bags by shaking bags for 24 hours in 50 ml of standard solutions made with known concentrations of KNO_3 or $(\text{NH}_4)_2\text{SO}_4$. Four bags were shaken individually in each of 7 values of total N ranging between 0 and 40 $\mu\text{g ml}^{-1}$. The range was based on maximum values found for ammonium and nitrate in resin bag eluates. The resin bags were squeezed free of excess standard solution, rinsed in distilled water, and shaken in 5% (v/v) HCl for 30 minutes. Eluates were analyzed for ammonium and nitrate as above and field results were then transformed according to the resultant calibration curves.

2.5 Soil Descriptions:

Soil samples for total N, total P, pH, organic matter, Na^+ , Mg^{++} , Ca^{++} , and K^+ were taken with a 7 cm deep, 5 cm diameter soil core directly adjacent to, but without disturbing, the resinbags. One core was taken per plot, air dried and sieved to 2 mm before analysis. Total N and total P were analyzed as above with 1 g and 2 g soil material, respectively. To measure pH, 20 g of soil material from each plot was shaken individually in 50 ml 0.01 M $\text{CaCl}_2 \cdot 2\text{H}_2\text{O}$ for 30 minutes. The resulting slurry was measured with a WTW (Germany) pH meter. Percent organic matter was quantified as percent mass lost after combustion of soils at 420°C in a muffle furnace. Soil samples were sent to Matrolab (Brakenfell, South Africa) for analysis of Na^+ , Mg^{++} , Ca^{++} , and K^+ using flame photometry and atomic absorption.

Soil moisture was recorded on each date that resin bags were switched by collecting approximately 40 g of soil into one screw-top glass vial per plot. Soils were weighed, oven dried at 105°C for 48 hrs and reweighed to obtain percent soil moisture. Soil temperature was also measured on each date that resin bags were switched in the acacia thicket and acacia cleared treatments only. A Fluke (USA) thermocouple was placed in the soil at 5 and 10 cm within each plot. Ambient air temperature was also recorded at this time.

2.6 Glasshouse Bioassay:

A soil bioassay was conducted using *Ehrharta calycina* to compare growth rates and N uptake of a weedy species on fynbos versus acacia soils. *E. calycina* was chosen due to its prominence in acacia stands that had been cleared one year earlier at Riverlands (personal observation). Although *E. calycina* is a native grass, it generally establishes on disturbed soils and is not an abundant plant in mature or early successional acid-sand lowland fynbos (Hoffman 1984, Hoffman *et al.* 1987). It is also considered an invasive alien in Australia, New Zealand, and the USA (Milberg and Lamont 1995).

Within each of the twelve plots in the fynbos and cleared acacia treatments, three sets of bulked soils were taken in mid October, giving 36 replicates per soil type. Each bulked sample was obtained by coring eight times with a 7 cm deep, 5 cm diameter core. In the fynbos, cores were taken in open spaces under the assumption that grasses would recruit away from large bush canopies and dense restio root mats. Soils were air dried, sieved to 2 mm, and placed into small plastic pots 10 cm high and 12 cm in diameter. Soil samples from each pot were analyzed for total N (as above) prior to the start of the bioassay experiment. These values were also used to compare total N from open space versus directly underneath canopies in the fynbos.

E. calycina seed, “mission” variety, was obtained from Agricol (Brackenfell, South Africa) and 10-20 individuals added to each pot. Starting in mid December, 1999 the pots were placed in a random formation within the glasshouse at the University of Cape Town and given 50 ml of tap water once every day to two days depending on weather conditions. One week after the seed germinated, pots were weeded such that only the largest individual grass shoot remained. Thereafter, all germinating seed shoots were pulled as soon as they were noticed. In February, 2000, after 45 days, plants were harvested, separated into root and shoot material, and washed free of remaining dirt. The material was dried at 80°C for at least 48 hrs, weighed, and ground with a Retsch (Germany) mixer mill. Due to the stunted growth of *E. calycina* on fynbos soils, the three replicates per plot were bulked before grinding to obtain sufficient material for total N analysis. Plant material was analyzed for total N as in Section 2.3.

2.7 Decomposition Microcosms:

To investigate the mechanistic link between quantitative N return and total N in the soil, potential rates of litter decomposition were compared between the fynbos, acacia, grass, and lupin treatments. A microcosm study was used to eliminate abiotic differences between treatments in the field. Although the rates can not be seen as representative of actual field rates, they serve as a comparative measure between treatments. The length of the experiment (one month) precludes the data from being representative of full litter decomposition, but rather measure the flush of soluble organic matter during the initial phase of decomposition. Rates of CO₂ release during soil microbe respiration was used as a measure of decomposition. All soil was taken from within the 12 fynbos plots using a 7 cm deep, 5 cm wide soil core, airdried, and shaken into one uniform mixture. Water holding capacity of the

fynbos soil mixture was measured by leaving thoroughly wetted soil in 3 glass funnels lined with filters and glass wool for three days to drain while capped to block evaporation. Percent moisture content of the soil mixture was measured by drying soils in an oven at 105°C for 48 hrs. The resultant water holding capacity, 19.23% (± 0.001), was then approximately halved to 10% to be used in the rest of the decomposition experiment.

Litter for this experiment was taken from the same samples used to determine litter total N (Section 2.3). For the fynbos treatment, fractions were mixed according to proportions for site 2. Each of the 6 microcosms per treatment received litter from one replicate plot.

Glass jars (100ml) with screw tops were filled with 50 g of air dried soil to which was added 5 ml of distilled water to achieve 10% water holding capacity. The lids were then placed over the jars, but not screwed on, so that CO₂ could escape but the moisture content stayed constant. The jars were left this way for 48 h so that the initial CO₂ spike caused by stimulation of the soil microbes with water would not be included in the data set. After 48 h the litter was added to the jars as a layer on top of the soil. For each of the four treatments plus one control (soil only), 6 replicates were used. A 10 ml screwtop vial with 8 ml of 0.6 M NaOH was placed without a top inside the jar and the jar sealed. Control vials of NaOH were used to account for CO₂ capture before the addition of the vials to the microcosm.

Vials were switched daily for one week and then on days 9, 11, 15, 19, 25, and 31.

Microcosms were kept in a 25°C incubation chamber for the duration of the experiment.

Vials of NaOH were kept sealed until they were titrated using a phenolphthalein indicator and HCl (Rowell 1994).

2.8 Statistics:

Data taken from the Riverlands natural experiment (sections 2.3, 2.4, and 2.5) were split into two general comparisons before being statistically analyzed: fynbos/acacia/cleared acacia and fynbos/grass/lupin. This was done to keep data from previously plowed land separate. All variables were analyzed using one-way nested analysis of variance (ANOVA) testing the effects of site within treatment. To use the monthly data in these analyses, mineralization data was summed per individual plot for annual totals. Soil moisture and temperature were averaged over the year for each individual plot. Monthly measured variables (mineralization, soil temperature, and soil moisture) were also analyzed with repeated measures ANOVAs. All data were \ln transformed to correct for normality and homoscedasticity except percentage data (soil moisture and organic matter) which were arcsine (squareroot) transformed. For all ANOVA analyses, post-hoc Tukey tests were used to determine significant differences between treatments.

In order to determine which soil and litter properties best explained differences in nitrogen fluxes, forward stepwise multiple regression models were used to ascertain which soil and litter properties best explained differences in nitrogen fluxes. The independent variables included the soil properties total N, total P, Ca^{++} , Mg^{++} , K^+ , Na^+ , organic matter, pH, and moisture content, plus litter P, and N return to the soil (litterfall x litter N content). Annual net NH_4^+ , NO_3^- , and total inorganic N mineralization were used as the dependant variables. Because highly related independent variables can lead to aberrant results (Zar 1984), a fully factorial correlation matrix was completed before the multiple regression analyses were done. Those independent variables which had the absolute value of the correlation coefficient greater than 0.80 ($|r| > 0.80$) were removed from the list and one was chosen to represent the related variables. The multiple regression analyses were completed including all treatments, and also for each separate treatment (fynbos, grass, lupin, acacia) in

an effort to see if different variables were responsible for driving N mineralization in different systems. After the initial results were obtained, regressions were re-analyzed with only those variables which had significant Beta values. Although this often resulted in a lower overall r^2 value (because less variance was explained), it resulted in higher degrees of significance for the overall regression.

Data from the bioassay experiment (Section 2.6) were compared using one-way ANOVAs with post-hoc Tukey tests and simple linear regression analysis. Where comparisons were made between samples that were bulked per plot ($n = 24$) and in which data was available for each individual replicate pot ($n = 72$), data were bulked per plot to give the appropriate sample size ($n = 24$).

Daily and cumulative CO_2 respiration rates from the decomposition microcosms (Section 2.7) were analyzed using repeated measures ANOVAs and post-hoc Tukey tests. Final cumulative CO_2 respiration was compared to litter quality (N and P) with simple linear regression analysis.

3 Results

3.1 Abiotic Factors:

Maximum and minimum average monthly temperatures (Fig 3.1.1 a, b) during the study period (25.58 ± 5.25 and $11.67 \pm 3.76^{\circ}\text{C}$, respectively), were similar to those averaged over the last 40 years (24.08 ± 5.10 and $12.27 \pm 3.35^{\circ}\text{C}$, respectively). Rainfall during the study period was 91.1 mm lower and peaked later than data averaged over the last 40 years (Fig 3.1.1 c, d).

Percent soil moisture content increased in all treatments in May, the first month in which the sample date occurred after the winter rains began (Fig 3.1.2 a, b). The soils remained moist until November, when the seasonal rain ended. Uncleared acacia stands had greater soil moisture than cleared stands, which, in turn, had greater soil moisture than fynbos (Fig 3.1.2 a). In the lupin, grass, fynbos comparison, only grass had significantly higher soil moisture content (Fig 3.1.2 b).

Soil temperatures at 5 cm depth in the two acacia treatments followed trends for maximum daily temperature (Fig 3.1.1 a and 3.1.2 c). Within the sampling period, both the maximum and minimum daily temperatures occurred in the cleared acacia stands, which reached levels of 31.75°C and 13.09°C respectively; these differences led to the cleared acacia plots having significantly different soil temperatures from uncleared plots (Fig 3.1.2 c).

3.2 Biomass, Litterfall, and N Return to Soil:

Litterfall was four times greater in acacia than fynbos plots (Fig 3.2.1 c). Acacia litter contained twice as much nitrogen and half as much phosphorus as the fynbos litter (Fig 3.2.1

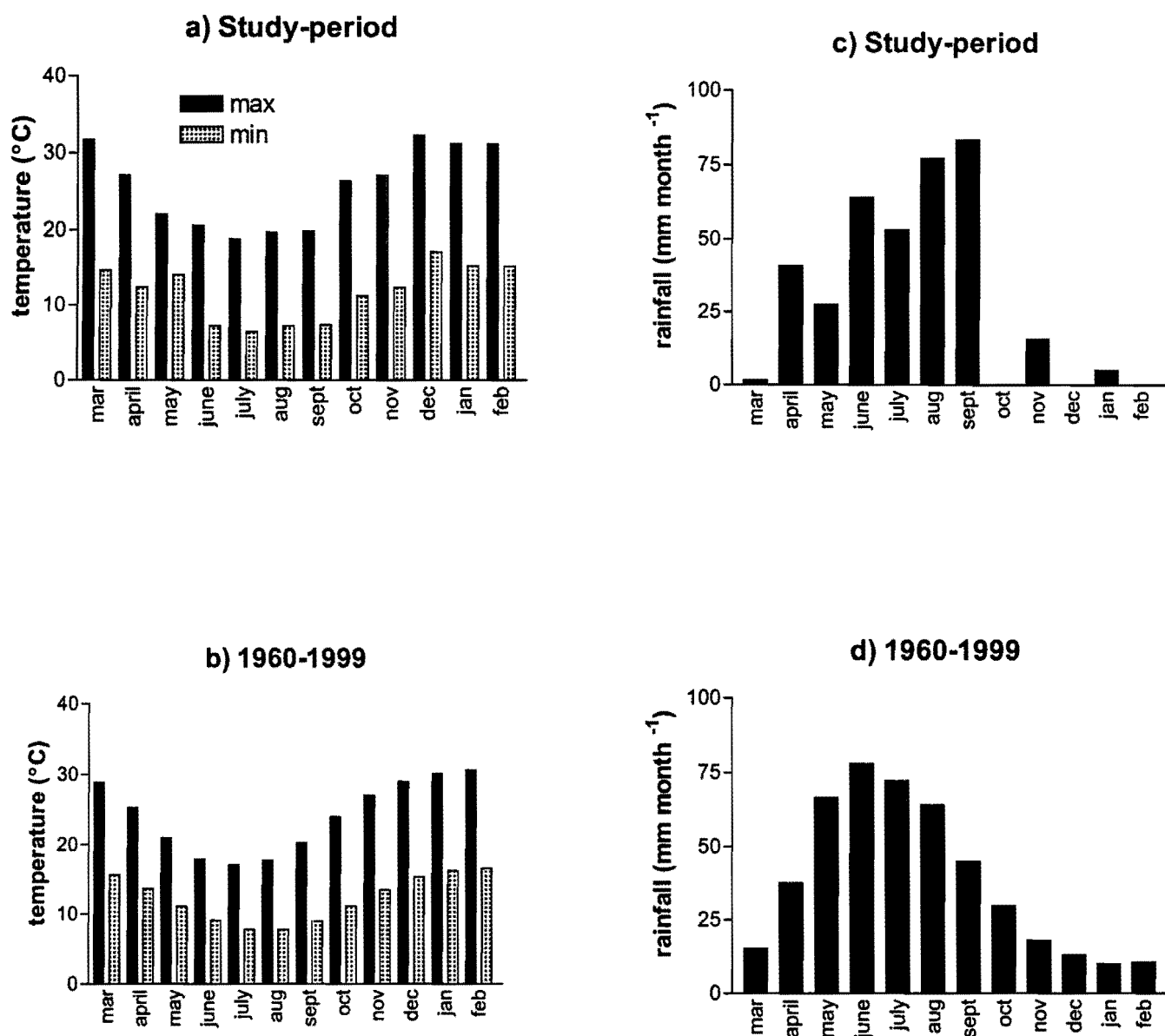


Figure 3.1.1. Malmesbury climate data: a) minimum and maximum daily temperature averaged for each month during the study period; b) minimum and maximum daily temperature averaged for each month between 1960 and 1999; c) monthly rainfall for the study period; d) monthly rainfall averaged for each month between 1960 and 1999. Data were obtained from the South African Weather Bureau.

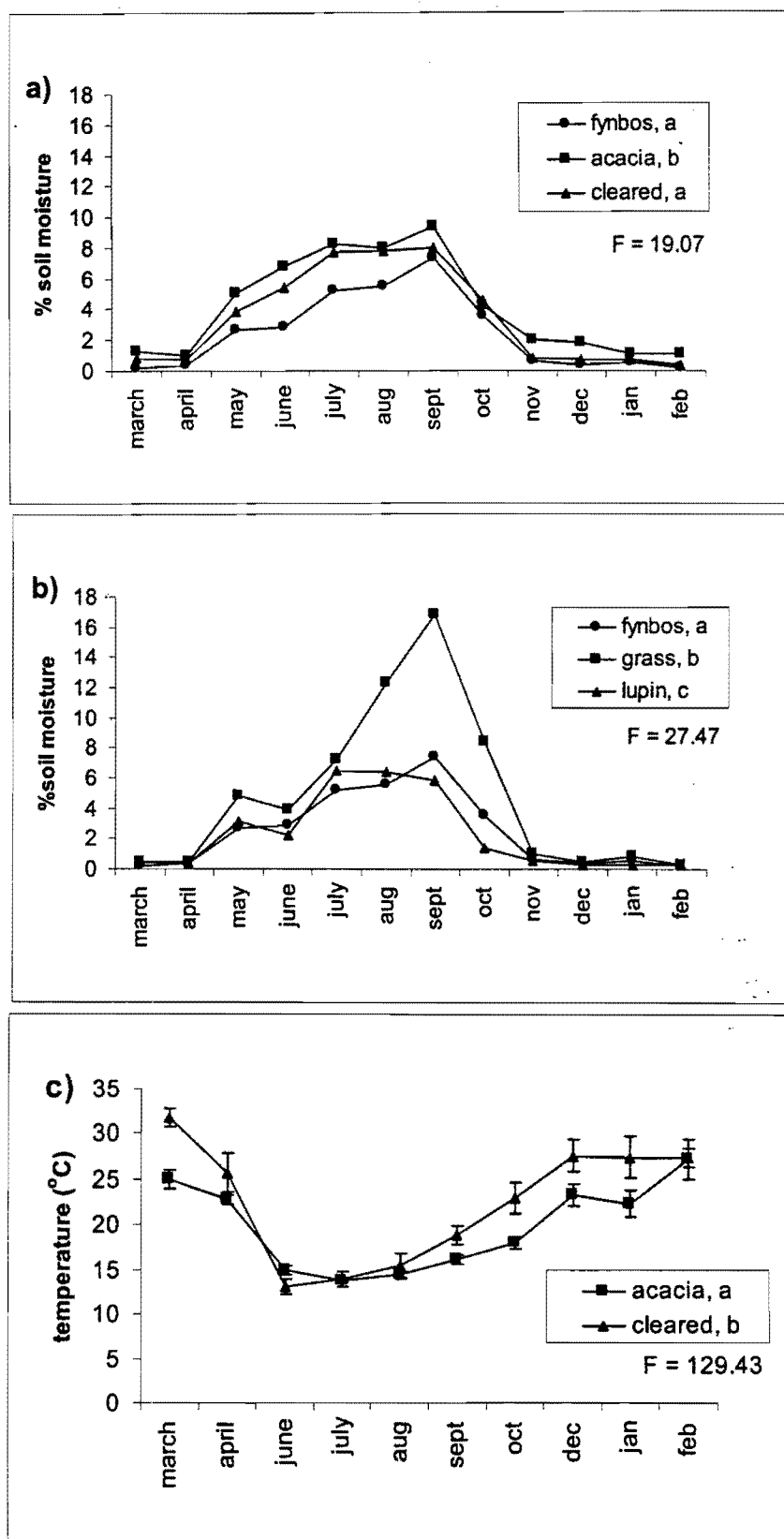
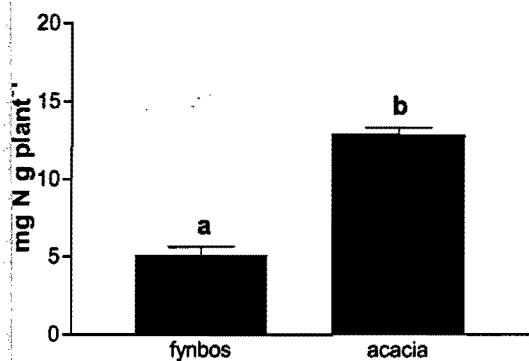
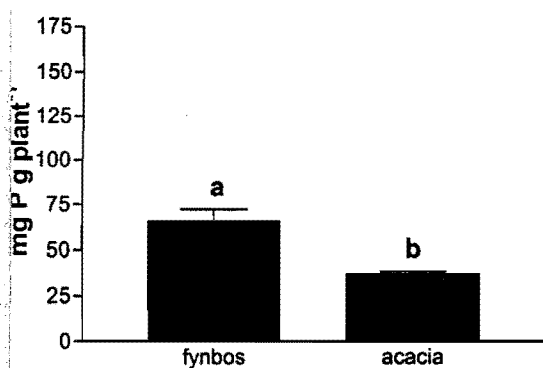


Figure 3.1.2. Monthly percent soil moisture content in a) fynbos, grass and lupin treatments, and b) fynbos, acacia, and cleared treatments and c) monthly soil temperatures in cleared and uncleared acacia treatments. Months run from March 1999 to February 2000. Temperature data were not obtained for May and November. F-values and letters (which denote significance at the level of $p \leq 0.05$) are from repeated measures one way ANOVAs with post hoc Tukey test comparisons.

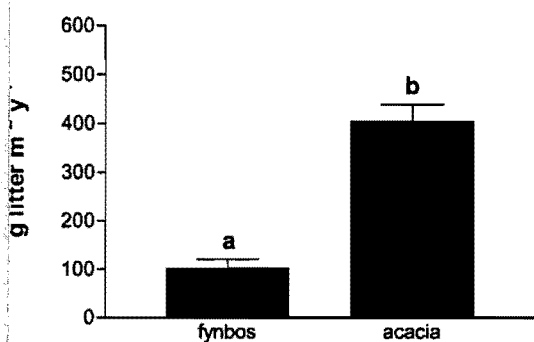
a) litter N



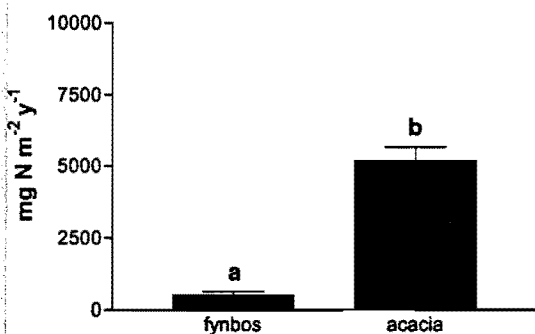
b) litter P



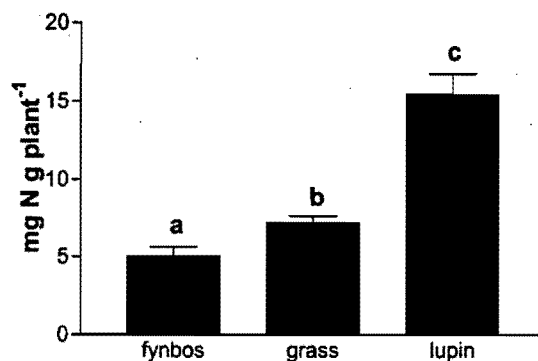
c) litterfall



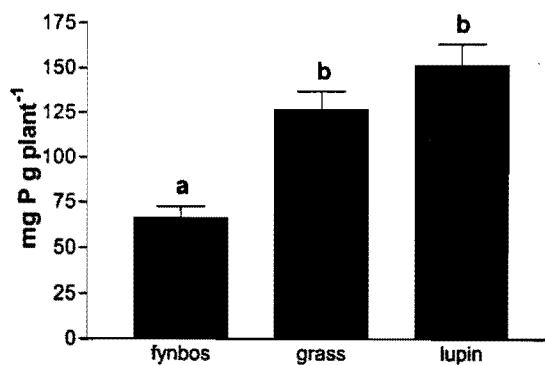
d) N return



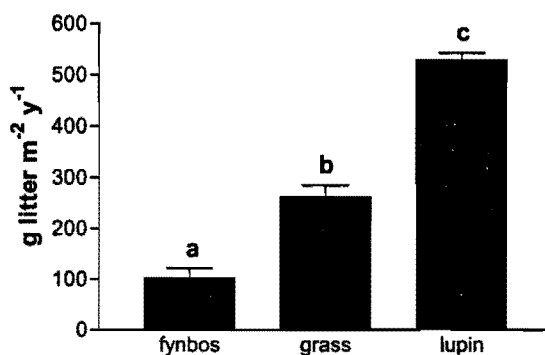
e) litter N



f) litter P



g) litterfall



h) N return

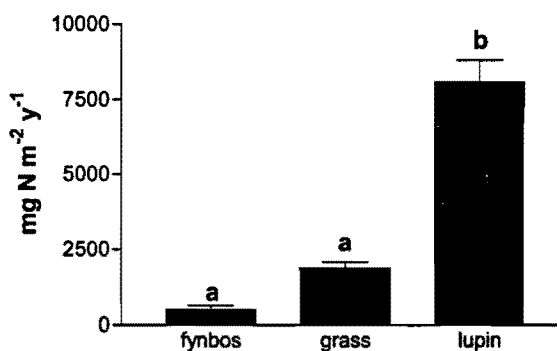


Figure 3.2.1. Litterfall and nutrient dynamics: a) litter N, b) litter P, c) litterfall, and d) nitrogen return to the soil (litter N x litterfall) for fynbos and uncleared acacia treatments and e) litter N, f) litter P, g) litterfall, and h) N return to soil for fynbos, grass, and lupin treatments. Letters represent significance at the level of $p \leq 0.01$. F values for one way nested ANOVAs can be found in Table 3.5.1.

a, b). Nitrogen return to the soil, (quantified as litterfall x litter N concentration), was ten times greater in acacia than fynbos plots (Fig 3.2.1 d).

Litterfall, litter nitrogen concentrations, and N return to the soil were higher in lupin than grass plots, which, in turn, were higher than fynbos plots (Fig 3.2.1 e, g, h). Litter phosphorus was higher in both lupin and grass litter than fynbos litter (Fig 3.2.1 f).

3.3 Net N Mineralization:

Calibration of resin bags showed that NH_4^+ was absorbed according to the equation: absorbed N = $0.74 \text{ actual N} + 0.41$ (Fig 3.3.1 a). In this equation, actual N represents the amount of N (g N ml^{-1}) with which the resin bag was treated and absorbed N represents the amount the N (g N ml^{-1}) measured in the HCl in which the resin bags were shaken (see Section 2.4). Similarly, NO_3^- was absorbed according to the equation: absorbed N = $0.44 \text{ actual N} + 0.38$ (Fig 3.3.1 b). For both NH_4^+ and NO_3^- , the resin bags did not absorb all ionic N in the surrounding solution. Rates of net N mineralization quantified from field resin bags were changed according to the above equations so that absorption efficiencies of the resin bags were accounted for.

Total annual net mineralized N was greater in both acacia treatments than in the fynbos, although there was no difference between cleared and uncleared acacia (Fig 3.3.2 c). The same patterns of significance held for annual net mineralized NH_4^+ and NO_3^- (Fig 3.3.2 a, b). Data plotted for each month show that the two acacia treatments pulsed in both forms of inorganic nitrogen with the first rains of the season (late April) and then dropped back to lower levels. Although net NH_4^+ mineralization was always detectable, net NO_3^- mineralization dropped to undetectable levels between January and April -- the hottest, driest time of the year (Fig 3.3.2 d, e, f and Fig 3.1.1 a, c).

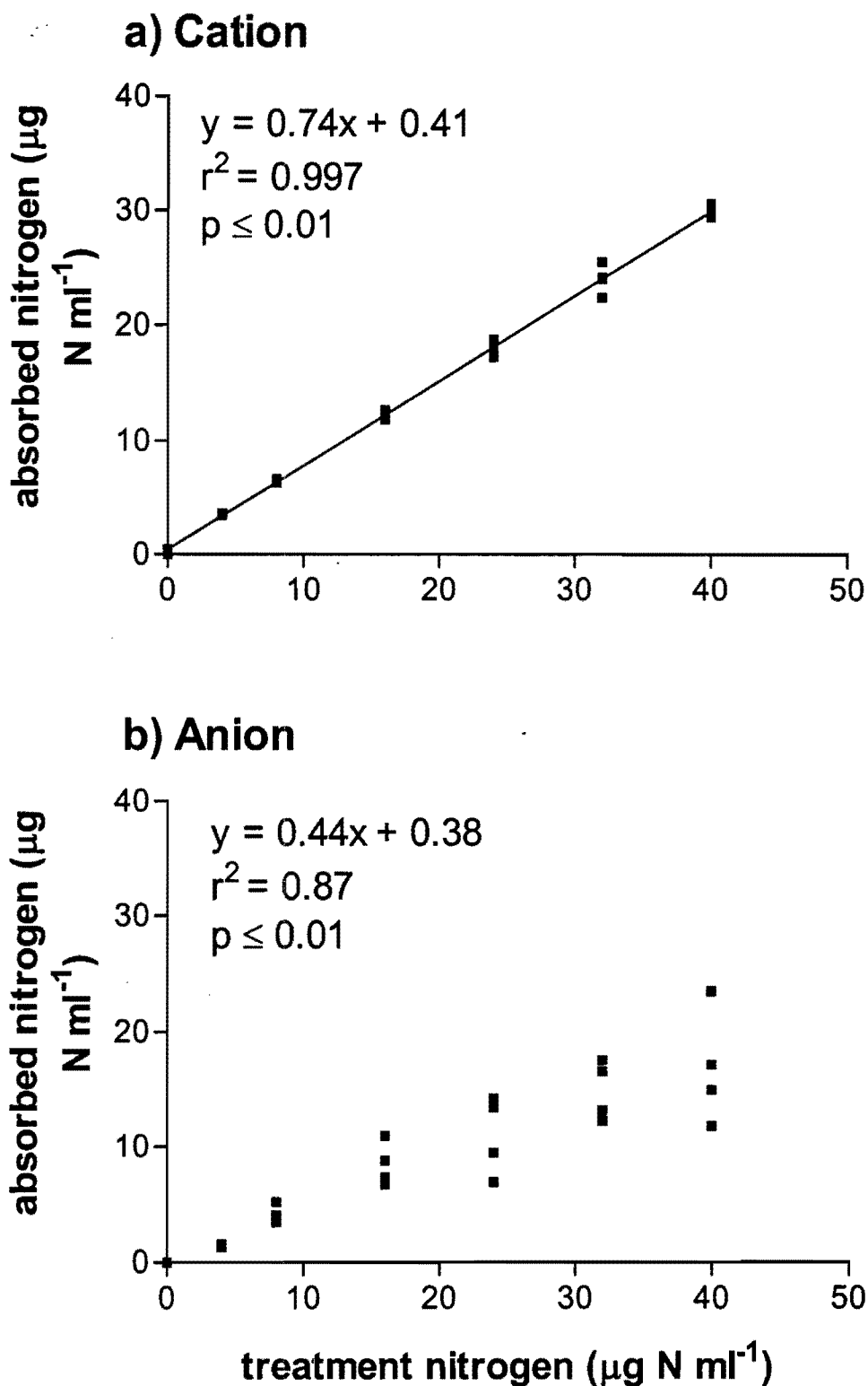


Figure 3.3.1. Calibration curves for a) cation and b) anion resinbags showing the relationship between the nitrogen concentrations of the treatment solutions and the actual absorption by the resin bags. See section 2.4 for methods. Best fit lines and statistics are from simple linear regression analysis.

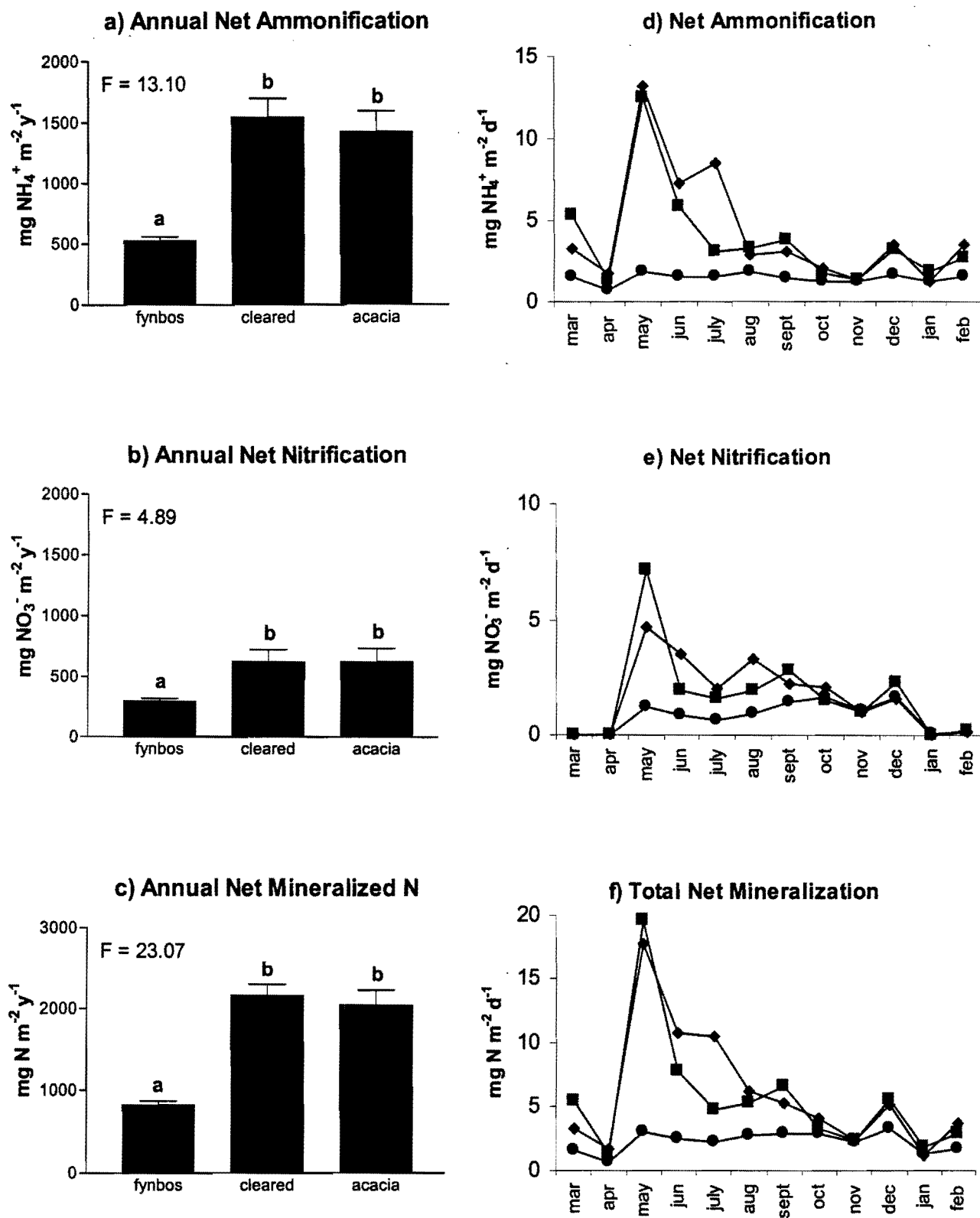


Figure 3.3.2. Fynbos, cleared, and uncleared acacia annual net mineralized a) NH₄⁺, b) NO₃⁻, and c) total inorganic N. F values and letters (which represent significance at the P ≤ 0.01 level) are from repeated measures ANOVAs of monthly data with post hoc Tukey tests to compare treatment effects. Monthly net mineralized d) NH₄⁺, e) NO₃⁻, and f) total inorganic N for fynbos (●), cleared (◆), and uncleared acacia (■). Months run from March 1999 to February 2000.

Annual net mineralized total N and NH_4^+ were higher in lupin than grass plots, which were higher than fynbos plots (Fig 3.3.3 a, c). Net mineralized NO_3^- was only significantly higher in lupin plots (Fig 3.3.3 b). The late April rains only caused a distinct pulse in the mineralized NO_3^- of the lupin plots (Figure 3.3.3d, e, f). Net NO_3^- mineralization, as above, dropped to undetectable levels between January and April in all three treatments (Fig 3.3.3 e).

3.4 Soil Descriptions:

Uncleared and cleared acacia soils were significantly higher ($p \leq 0.01$) than fynbos soils in total N, organic matter, Ca^{++} , Mg^{++} , and K^+ (Fig 3.4.1 a-d, f). Total P and pH showed no differences between treatments (Fig 3.4.1 e, h). Na^+ was higher in uncleared than cleared acacia stands, but fynbos soils were not significantly different than either (Fig 3.4.1 g).

Grass and lupin soils were higher than fynbos soils in total N, Ca^{++} , Mg^{++} , and pH (Fig 3.4.2 a, b, f, h). K^+ was higher in lupin than fynbos soils, but grass plots were not significantly different than either treatment (Fig 3.4.2 c). Soil organic matter and total P were both higher in grass than fynbos or lupin plots (Fig 3.4.2 d, e). Na^+ was higher in grass than fynbos soils, but lupin plots were not significantly different from either (Fig 3.4.2 g).

The correlation matrix for independent variables used in the multiple regression analysis (Table 3.4.1) showed that soil N, organic matter, Ca^{++} , and Mg^{++} were highly related ($|r| > 0.80$). Both soil N and organic matter represent the pool of organic nutrients, but soil N is more likely to explain rates of change from organic to inorganic N (i.e., mineralization), so it was chosen to represent both variables in the regression analysis. Ca^{++} and Mg^{++} are both divalent cations that are related to the ion exchange capacity of the soil; Mg^{++} was arbitrarily chosen to represent the two.

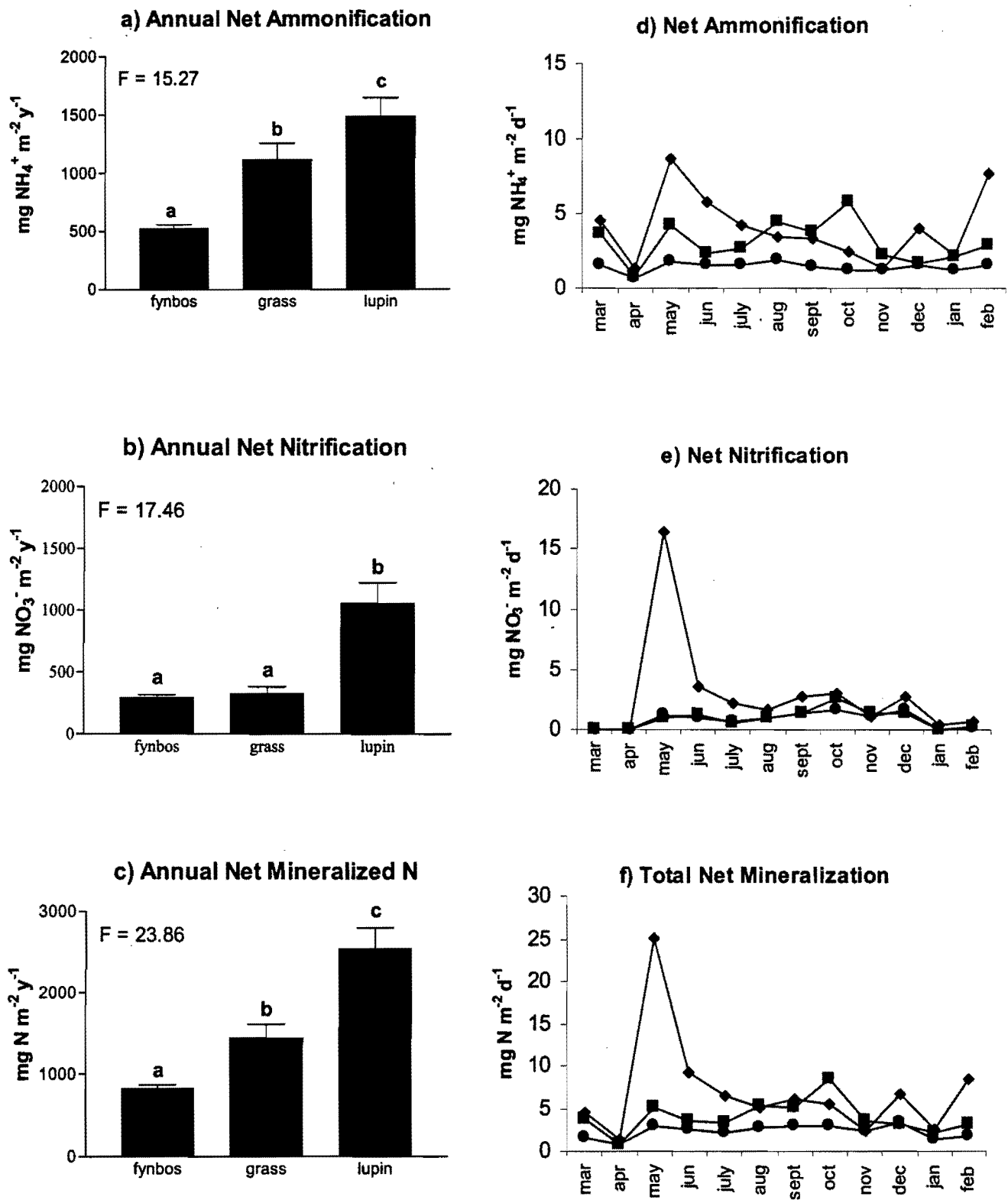


Figure 3.3.3. Fynbos, grass, and lupin annual net mineralized a) NH_4^+ , b) NO_3^- and c) total inorganic N. F values and letters (which represent significance at $P \leq 0.01$) are from repeated measures ANOVAs of monthly data with post hoc Tukey tests to compare treatment effects. Monthly net mineralized d) NH_4^+ , e) NO_3^- and f) total inorganic N for fynbos (\bullet), grass (\blacksquare), and lupin (\blacklozenge). Months run from March 1999 to February 2000.

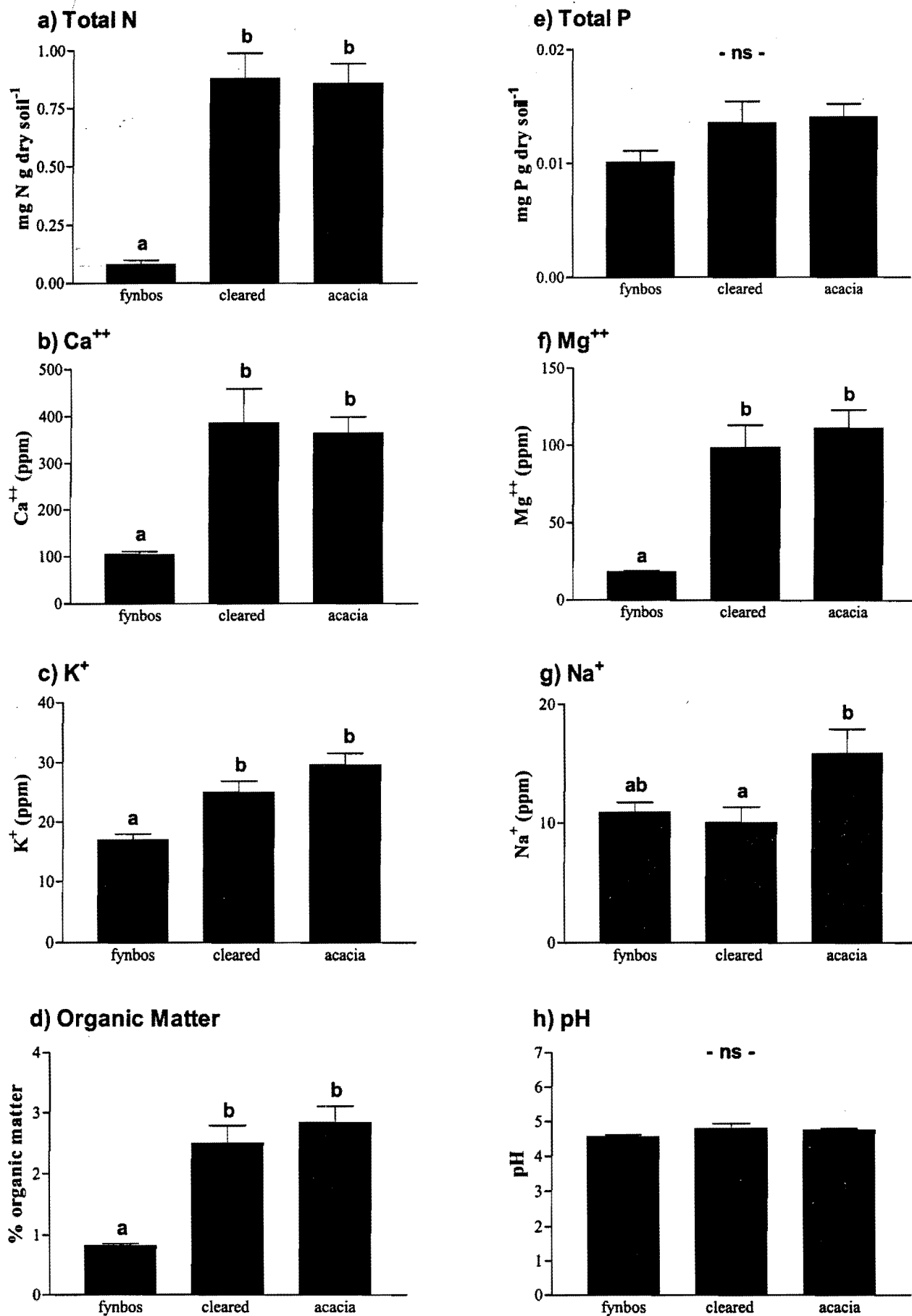


Figure 3.4.1. Soil a) total N, b) Ca⁺, c) K⁺, d) % organic matter, e) total P, f) Mg⁺, g) Na⁺, and h) pH for fynbos, cleared, and uncleared acacia treatments. Letters represent significance at the P ≤ 0.01 level for post hoc Tukey tests. F-values for one way nested ANOVA's can be found in Table 3.5.1.

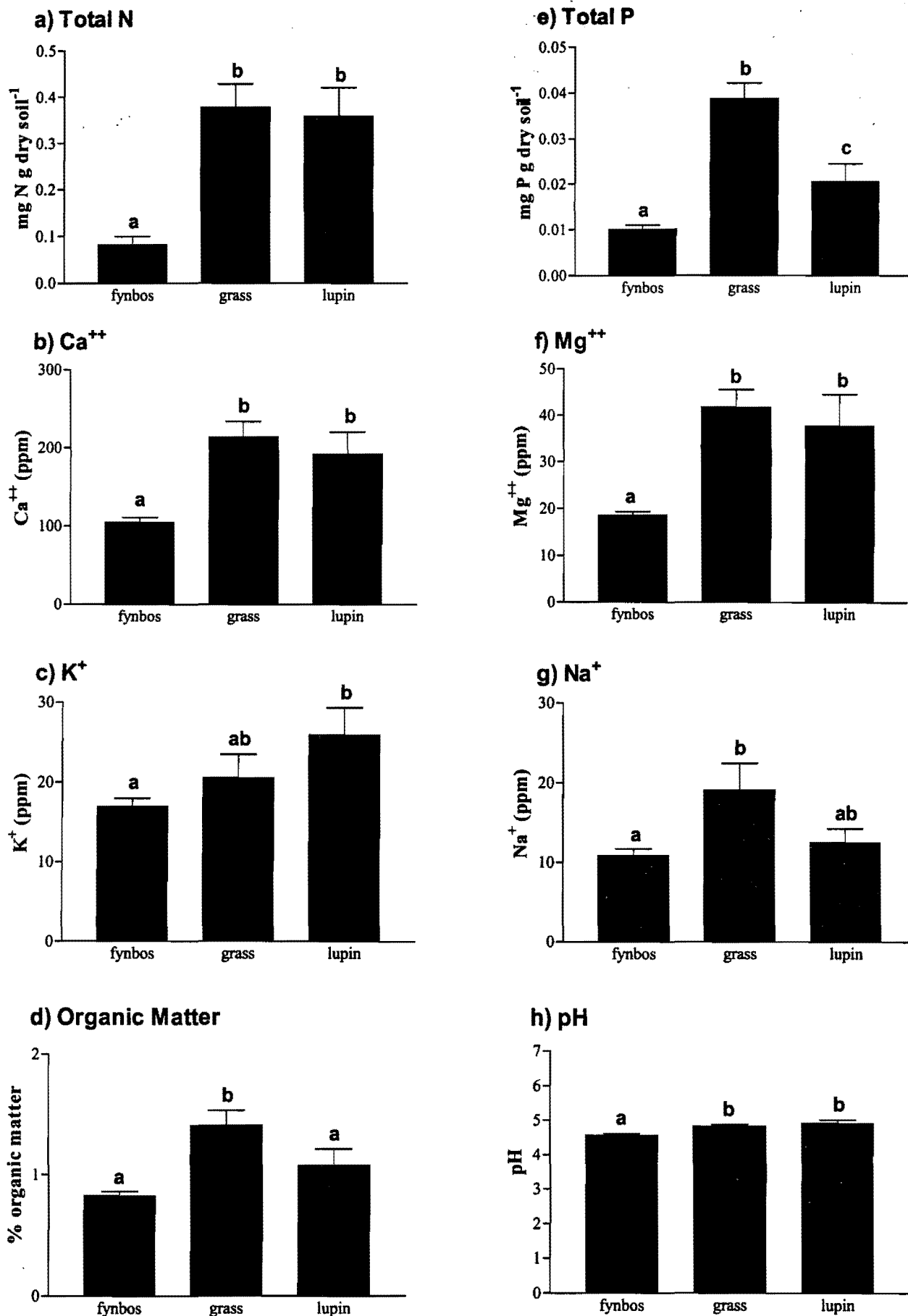


Figure 3.4.2. Soil a) total N, b) Ca⁺, c) K⁺, d) % organic matter, e) total P, f) Mg⁺, g) Na⁺, and h) pH for fynbos, grass, and lupin treatments. Letters represent significance at the $P \leq 0.01$ level for post hoc Tukey tests. F-values for one way nested ANOVAs can be found in Table 3.5.1.

Table 3.4.1. R values for correlations between independent variables of the forward stepwise regression analyses. Data was taken from fynbos, grass, lupine, and uncleared acacia plots (n = 24). Monthly soil water data was averaged for each plot over the year of the study. Bold values, with $|r| > 0.80$, were considered “highly related”. * $p \leq 0.05$.

	Soil N	Soil P	Litter P	OM	pH	Ca ⁺⁺	Mg ⁺⁺	K ⁺	Na ⁺	Soil water	N re-turn
Soil N	1	0.10	0.37*	0.96*	0.22	0.95*	0.95*	0.69*	0.45*	0.59*	0.39*
Soil P		1	0.43*	0.02	0.20	0.10	-0.04	0.14	0.43*	0.42*	-0.01
Litter P			1	-0.49*	0.39*	-0.32*	-0.45*	-0.15	-0.03	-0.27	0.36*
OM				1	0.10	0.93*	0.97*	0.65*	0.44*	0.62*	0.25
pH					1	0.35*	0.18	0.42*	0.31*	0.11	0.49*
Ca ⁺⁺						1	0.95*	0.72*	0.44*	0.56*	0.35*
Mg ⁺⁺							1	0.65*	0.42*	0.55*	0.31*
K ⁺								1	0.53*	0.30*	0.44*
Na ⁺									1	0.62*	0.05
Soil water										1	-0.13
N re-turn											1

Forward stepwise multiple regressions were used to choose those variables which best explained the variance in mineralization rates. The most general model, annual net N mineralized across all treatments, showed that N return to the soil and soil moisture explained 63% of the variance in mineralization rates (Table 3.4.2 c). Soil N (Beta = 0.78, $r^2 = 0.61$, $p = 0.003$), soil moisture (Beta = 0.73, $r^2 = 0.53$, $p = 0.007$), and Mg^{++} (Beta = 0.76, $r^2 = 0.58$, $p = 0.004$) were all significant variables for the grass treatment by themselves, but not in any combination together. Soil N was considered the most important as it led to the highest r^2 and lowest p values for the regression. The only significant variable for the acacia treatment was soil moisture.

Annual net NH_4^+ mineralization across all treatments showed soil N, N return, Mg^{++} , and soil moisture accounting for 58% of the variability (Table 3.4.2 a). The only significant variable for the grass treatment was soil moisture. The acacia treatment model included soil moisture, N return, Mg^{++} , and soil P to explain 87% of the variability.

Annual net NO_3^- mineralization across all treatments was best explained by N return, which explained 46% of the variability (Table 3.4.2 b). The model for the grass treatment included the variables Mg^{++} , N return, pH, soil N, and soil moisture to account for 93% of the variability in NH_4^+ mineralization. The lupin model included soil P, N return, Na^+ , and pH to account for 87% of the variability. The acacia treatment only included the cations Mg^{++} and Na^+ and explained 56% of the variability.

The fynbos treatment was never found to have significant variables correlating with net mineralization rates and the lupin treatment had no significant variables for the net NH_4^+ and net total N mineralization rates.

Table 3.4.2. Multiple forward stepwise regressions for the dependant variables a) annual net NH_4^+ mineralization, b) annual net NO_3^- mineralization, and c) annual net total inorganic N mineralization for all treatments (n = 48), fynbos only (n = 12), grass only (n = 12), lupin only (n = 12), and acacia only (n = 12). Possible independent variables included soil N, soil P, litter P, soil pH, soil K^+ , soil Na^+ , soil Mg^{++} , soil moisture (H_2O), and N return (litterfall x litter N). * $p \leq 0.05$, ** $p \leq 0.01$.

a)	All		Fynbos		Grass		Lupin		Acacia	
step	variable	Beta	variable	Beta	variable	Beta	variable	Beta	variable	Beta
1	soil N	0.96*			soil H_2O	0.76**			soil H_2O	1.68**
2	N return	0.49**							N return	0.42**
3	Mg^{++}	-0.79*							Mg^{++}	-1.11**
4	soil H_2O	0.31*							soil P	0.44**
r^2	0.58**		No significant variables		0.60**		No significant variables		0.87**	

b)	All		Fynbos		Grass		Lupin		Acacia	
step	variable	Beta	variable	Beta	variable	Beta	variable	Beta	variable	Beta
1	N return	0.68**			Mg^{++}	1.70**	soil P	0.35*	Mg^{++}	1.14**
2					N return	-0.67**	N return	0.68**	Na^+	-0.98**
3					pH	-0.84**	Na^+	0.46**		
4					soil N	-0.90**	pH	-0.43*		
5					soil H_2O	0.54*				
r^2	0.46**		No significant variables		0.93**		0.87**		0.56**	

c)	All		Fynbos		Grass		Lupin		Acacia	
step	variable	Beta	variable	Beta	variable	Beta	variable	Beta	variable	Beta
1	N return	0.79**			soil N	0.78**			soil H_2O	0.69**
2	soil H_2O	0.30**								
r^2	0.63**		No significant variables		0.61**		No significant variables		0.48**	

3.5 Site Effects:

Because each of the five treatments (fynbos, grass, lupin, cleared and uncleared acacia) was split into three separate sites within the reserve, nested ANOVA's (with site nested in treatment) were used to analyze data. Although site effects were often significant, there is only one instance (for soil pH in the fynbos, cleared, and uncleared acacia comparison) of site having a significant effect without treatment also having a significant effect (Table 3.5.1).

In order to elucidate controls on N cycling in native systems, significant differences between sites were only investigated further within the fynbos treatment (Table 3.5.2). Annual net total N mineralization was higher in site 3 than site 2. While annual net NO_3^- mineralization did not differ between sites, annual net NH_4^+ mineralization was higher in sites 1 and 3 than in site 2. Litter N and P were higher in site 3 than sites 1 or 2. Litterfall was greater in site 2 than site 1. N return from aboveground biomass to the soil was lowest in site 1. Total soil N and P, % organic matter, Mg^{++} , K^+ , and Na^+ did not differ between sites.

Fynbos litter differed in the proportion of its constituent parts depending on the site from which it came. *P. scolymocephala* litter consisted only of leaves, 4-5 y old *L. parile* contained 58% leaves and 42 % flowers while 5-6 y old *L. parile* contained 92% leaves and 8% flowers (Table 3.5.3).

3.6 Glasshouse Bioassay:

The soils collected for use in the glasshouse bioassay experiment showed that total N was higher in soils from acacia stand than from fynbos soils (Fig 3.6.1 a). After 45 days of growth, total, shoot, and root biomass of *E. calycina* was greater for plants grown on acacia than fynbos soil (Fig 3.6.1 e, f, g). Root:shoot ratios were greater for plants grown on fynbos soils (Fig 3.6.1 c). The total nitrogen content of each plant shoot (aboveground biomass) was

Table 3.5.1. F-values for nested one way ANOVAs (site nested within treatment). All data were ln transformed except %OM, which were arcsine (squareroot) transformed. Total N plant, total P plant, annual litterfall, and N return data were not collected for cleared acacia plots. Soil temperature at 5 cm was only collected for acacia and cleared acacia treatments. Monthly collected soil temperature and moisture data were averaged over the 12 months for each plot. * $p \leq 0.01$.

<i>Dependant Variable</i>	<i>fynbos/cleared/acacia</i>		<i>fynbos/grass/lupin</i>	
	<i>treatment</i>	<i>site</i>	<i>treatment</i>	<i>site</i>
Annual NH_4^+ min	64.53*	5.32*	36.44*	3.35*
Annual NO_3^- min	8.52*	2.61	27.25*	2.25
Annual total N min	106.04*	6.97*	45.88*	3.70*
Total soil N	76.05*	3.24	29.21*	5.71*
Total soil P	2.56	1.26	23.45*	0.89
% Soil moisture	47.97*	8.12*	56.51*	7.12*
Soil temp at 5 cm	156.52*	2.15	N/A	N/A
% OM	29.50*	2.92	14.53*	6.15*
pH	3.97	4.68*	7.88*	1.69
Ca^{++}	39.63*	3.23	23.59*	3.49*
Mg^{++}	98.47*	2.86	38.35*	7.89*
K^+	23.00*	2.04	4.90	1.75
Na^+	5.00*	0.81	5.03*	1.51
N litter	226.45*	10.78*	58.53*	3.05
P litter	105.06*	13.12*	62.61*	6.98*
Annual litterfall	186.68*	13.43*	169.68*	2.47
N return	174.16*	6.76*	86.40*	0.94

Table 3.5.2. Site differences within the fynbos treatment. Letters represent differences based on one way ANOVAs with post hoc Tukey tests ($n = 4$, $p \leq 0.05$).

Variable	SITE 1	SITE 2	SITE 3
Species (age)	<i>P. scolymocephela</i> (4-5 y)	<i>L. parile</i> (4-5 y)	<i>L. parile</i> (5-6 y)
community	Resteoid dominated	Proteoid dominated	Proteoid dominated
Annual NH_4^+ min ($\text{mg N m}^{-1} \text{y}^{-1}$)	559.4 ± 12.7 a	415.5 ± 45.5 b	609.8 ± 40.5 a
Annual NO_3^- min ($\text{mg N m}^{-1} \text{y}^{-1}$)	295.2 ± 27.1	263.9 ± 39.8	329.4 ± 43.0
Annual total N min ($\text{mg N m}^{-1} \text{y}^{-1}$)	854.6 ± 29.6 ab	679.4 ± 84.7 b	939.2 ± 63.2 a
Total soil N (mg N g soil^{-1})	0.08 ± 0.03	0.05 ± 0.03	0.12 ± 0.02
Total soil P (mg P g soil^{-1})	0.008 ± 0.001	0.012 ± 0.001	0.010 ± 0.001
Average annual % soil water	3.1 ± 0.1 a	2.2 ± 0.1 b	2.1 ± 0.1 b
% OM	0.82 ± 0.06	0.80 ± 0.07	0.85 ± 0.07
pH	4.43 ± 0.04 a	4.61 ± 0.04 ab	4.74 ± 0.19 b
Ca^{++} (ppm)	87.0 ± 5.6 a	102.8 ± 6.4 ab	125.8 ± 9.3 b
Mg^{++} (ppm)	17.8 ± 0.8	17.3 ± 0.5	20.8 ± 1.8
K^+ (ppm)	19.3 ± 1.6	17.3 ± 1.7	14.5 ± 1.3
Na^+ (ppm)	10.8 ± 1.8	11.8 ± 2.0	10.3 ± 0.5
N litter (mg N g plant^{-1})	4.1 ± 0.2 a	3.5 ± 0.2 a	7.5 ± 0.9 b
P litter ($\mu\text{g P g plant}^{-1}$)	49.8 ± 1.4 a	55.5 ± 1.0 a	92.9 ± 9.1 b
Litterfall ($\text{g m}^{-2} \text{y}^{-1}$)	37.2 ± 9.5 a	155.1 ± 27.8 b	114.5 ± 26.2 ab
N return ($\text{mg N m}^{-2} \text{y}^{-1}$)	151.5 ± 37.6 a	534.7 ± 79.3 b	863.1 ± 241.8 b

Table 3.5.3. Proportions of each litter fraction for the three fynbos sites separately and averaged across sites for the other treatments. Seeds were not included as they do not decompose and add to the nutrient pools of soils.

<i>Treatment</i>	<i>Site</i>	<i>Fraction</i>	<i>Proportion of total biomass</i>
Fynbos	P. scolymocephela (4-5 y)	Leaves	1.00
	L. parile (4-5 y)	Leaves	0.58
		Flowers	0.42
	L. parile (5-6 y)	Leaves	0.92
		Flowers	0.08
Grass	All	Standing dead	1.00
Lupin	All	Non-lupine dead	0.46
		Lupine vegetative	0.44
		Lupine pods	0.10
Acacia	All	Leaves (phyllodes)	0.60
		Pods	0.22
		Flowers	0.18

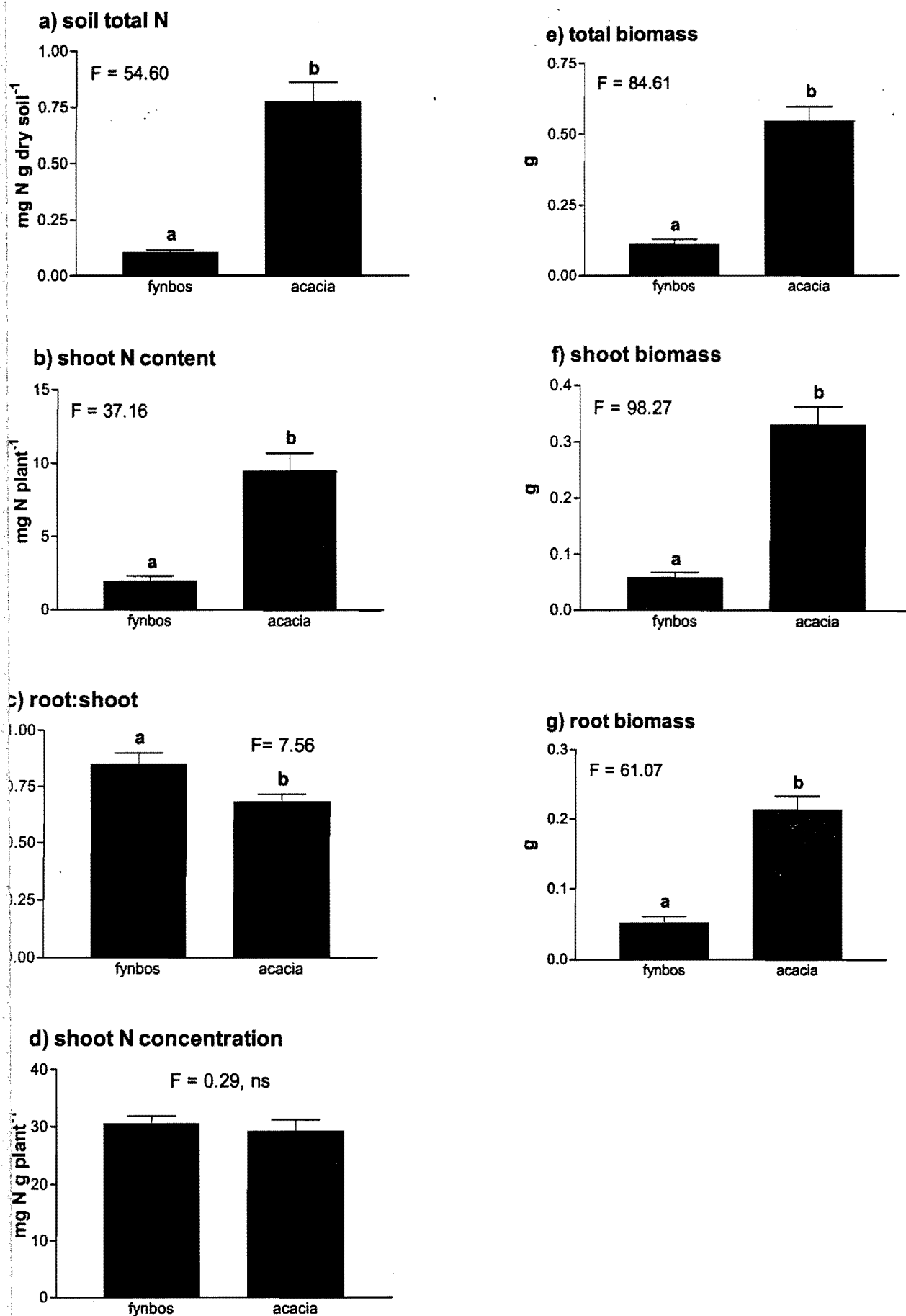


Figure 3.6.1. Bioassay experiment using *Ehrharta calycina* on soils from fynbos and acacia stands. Letters denote significance at the level of $p \leq 0.01$ using one way ANOVAs for a) soil total N, and *E. calycina* b) shoot N content, c) root:shoot ratios, d) shoot N concentrations, e) total biomass, f) shoot biomass, and g) root biomass.

greater on plants from acacia soils (Fig 3.6.1 b) although shoot N concentration (shoot N content/ shoot biomass) was not significantly different between soil types (Fig 3.6.1 d).

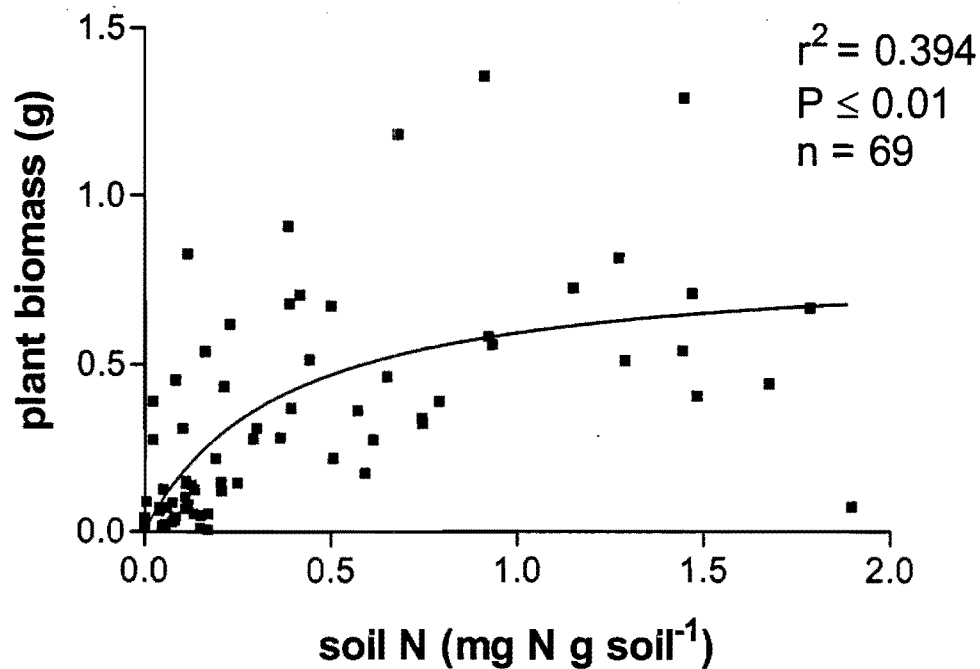
Soil organic nitrogen for each pot accounted for 39% of the variability in *E. calycina* total biomass after 45 days of growth, showing a logistic growth curve. When the data were bulked per plot, soil organic nitrogen accounted for 56% of the variability in the shoot N content of *E. calycina* (Fig 3.6.2 a, b).

Soils for the bioassay experiment were taken from the spaces between plants in the fynbos, whereas soils for descriptive plot characteristics (Section 3.4) were taken from underneath the canopies of *L. parile* or *P. scolymocephala*. Comparisons of the total N content showed that there was no significant difference in N levels between soils from open areas (0.10 ± 0.06 mg N g dry soil⁻¹) and underneath canopies (0.08 ± 0.06 mg N g dry soil⁻¹), (one way ANOVA, $F = 1.935$, $p = 0.18$).

3.7 Decomposition Microcosms:

Soil respiration (which was used as a proxy for initial rates of litter decomposition) peaked 2 days after the litter was added to the soil for all treatments except acacia; acacia litter decomposition peaked on day 3 (Figure 3.7.1 a). Thereafter, the daily decomposition rate slowly declined in all treatments until the end of the experiment (day 31). Daily rates and cumulative amounts of CO₂ respiration showed that lupin litter decomposed the fastest (Figure 3.7.1 a, b). Although acacia, grass, and fynbos litter decomposed faster than controls, they were not significantly different from each other.

a) biomass vs. soil N



b) plant N vs. soil N

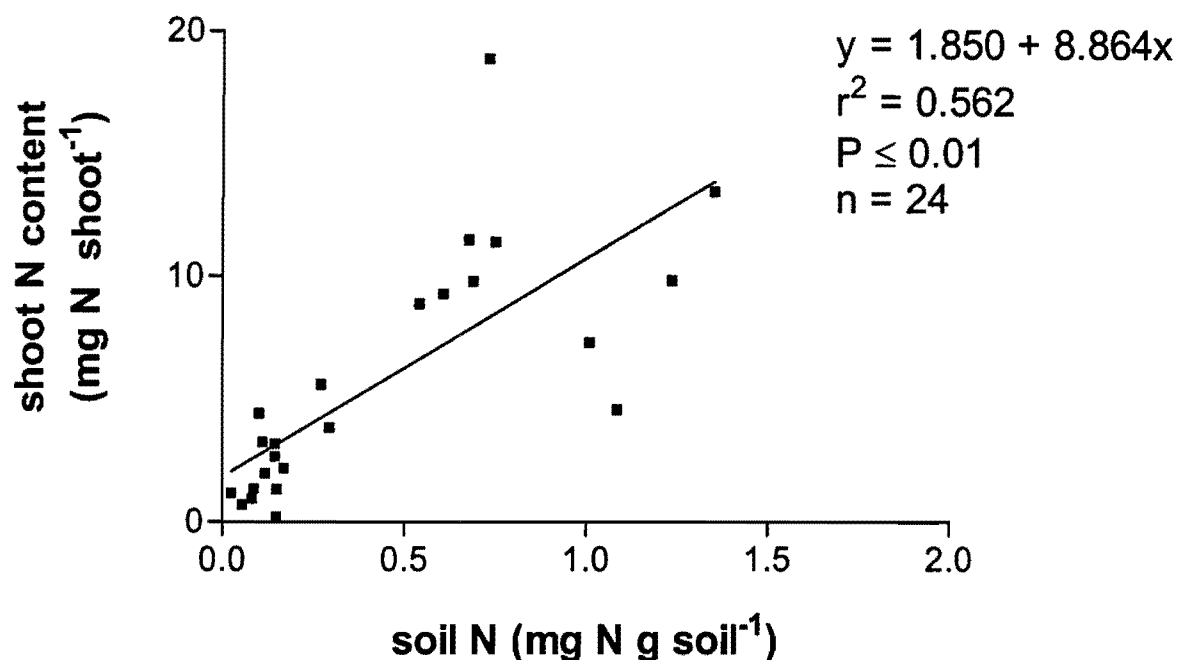


Figure 3.6.2. a) The non-linear regression curve describing the growth of *Ehrharta calycina* after 45 days as a function of soil organic N and b) the linear equation describing whole shoot N content as a function of soil organic N.

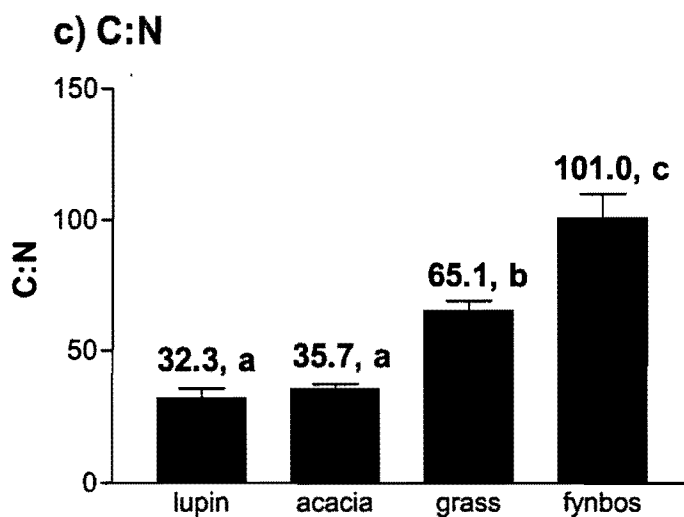
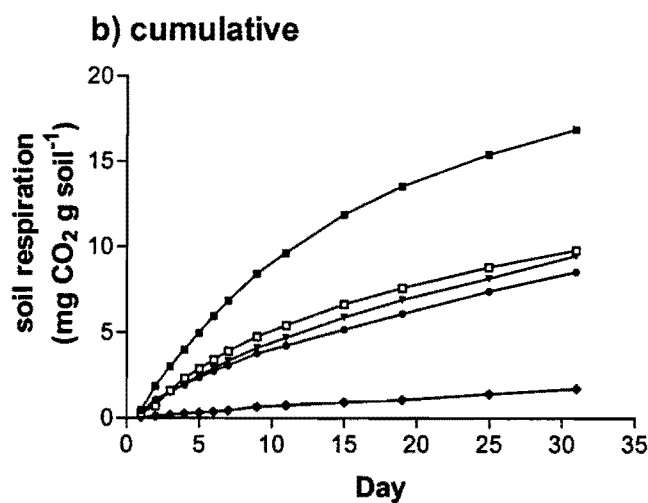
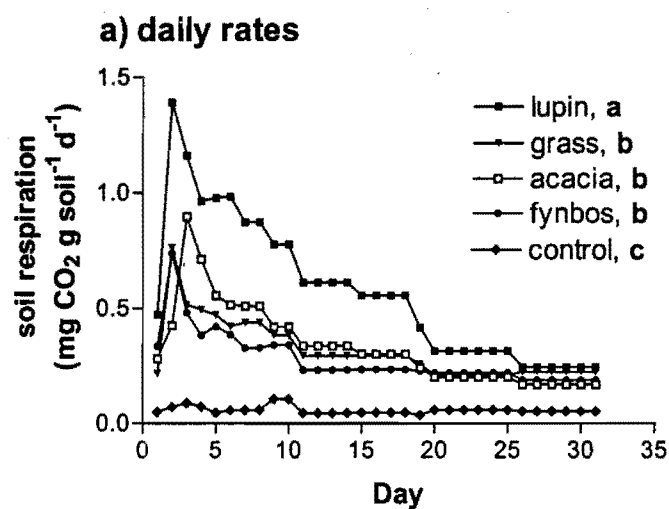
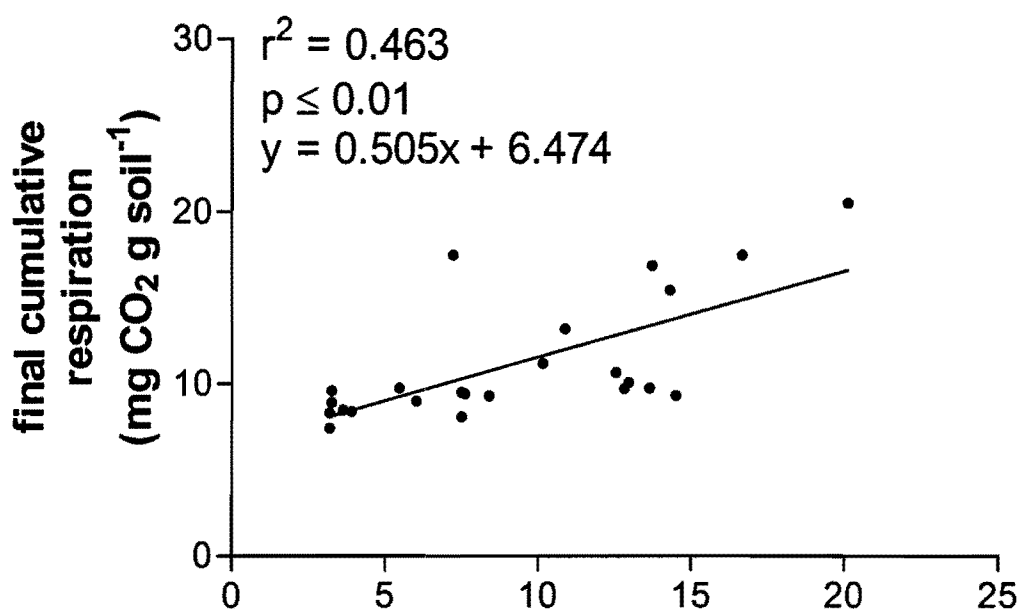


Figure 3.7.1. Soil respiration within litter decomposition microcosms expressed as a) a daily rate (mg CO₂ g soil⁻¹ d⁻¹) and b) cumulatively (mg CO₂ g soil⁻¹) for each day. Letters indicate significant differences between treatments in daily rate data (repeated measures ANOVA: $F = 111.22$, $p \leq 0.01$; post hoc Tukey test: $p \leq 0.01$). c) C:N ratios in each litter type with means written above bars. Letters represent significant differences from a one way ANOVA: $F = 35.90$, $p \leq 0.01$; post hoc Tukey test: $p \leq 0.01$.

Lupin and acacia litter had similar C:N ratios, 32.3 and 35.7, respectively (Fig 3.7.1 c). Grass litter had a significantly higher ratio than acacia or lupin, and fynbos had a higher ratio than grass litter. Litter phosphorus accounted for 38% of the variability in final cumulative CO₂ respiration (data not shown, $p \leq 0.01$) while litter nitrogen accounted for 46% of the variability (Figure 3.7.2 a). Investigation of outlying data points in the relationship between litter N and respiration showed that the acacia treatment had consistently lower respiration rates than expected by its high N content. When these points were removed from the correlation, litter N accounted for 76% of the variability in final cumulative CO₂ respiration (Figure 3.7.2 b) but litter P accounted for no more variability in the data than before (data not shown, $r^2 = 0.39$, $p \leq 0.01$).

a) all treatments



b) without acacia

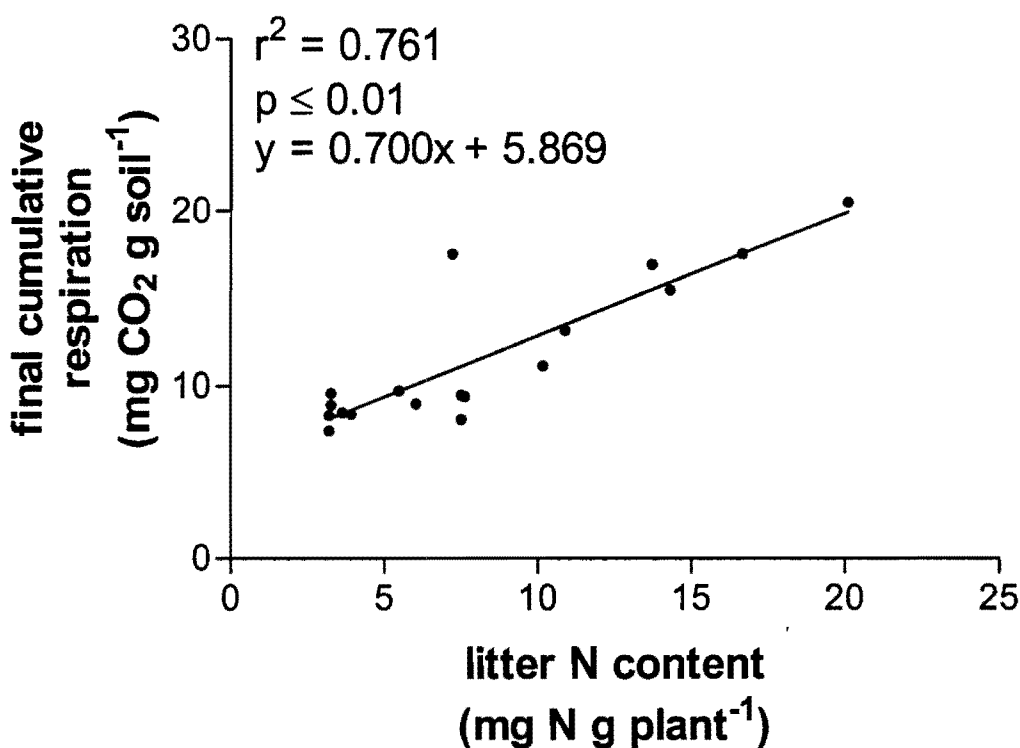


Figure 3.7.2. Final cumulative soil respiration (mg CO₂ g soil⁻¹) as a function of the nitrogen content (mg N g plant⁻¹) of litter used in decomposition microcosms a) with all treatments and b) with all treatments excluding acacia.

4 Discussion

4.1 N Cycling in the Fynbos:

Small changes in soil characteristics can have large effects on native fynbos species composition (Richards *et al.* 1997 a, b), making understanding the drivers of N cycling within the ecosystem important. Choosing different sites within the fynbos treatment at Riverlands presented the opportunity to look at controls of N in soils. Site 1 was a Restionaceae dominated community with *P. scolymocephala* as the target shrub. Sites 2 and 3 were proteoid dominated communities of different ages with *L. parile* as the target species (Table 3.5.2). By comparing sites 1 and 2, the effects of species composition could be investigated without the confounding effects of stand age. By comparing sites 2 and 3, the effects of stand age could be investigated without the confounding effects of species composition.

In the comparison of sites 1 and 2 for species effects, the most evident difference was the higher soil moisture in site 1. Higher levels of moisture probably led to the higher NH_4^+ mineralization rates in site 1 and may have directly or indirectly led to the species differences between the sites. It should be noted that the two sites had similar soil parent materials, soil N, organic matter, and litter N content. From the litterfall and N return data, which were both four times greater in site 2, it would be expected that the N mineralization pattern would be the opposite. The lower N mineralization in site 2 despite larger N inputs, however, stresses the importance of soil moisture. Similarly, Stock and Lewis (1988) found that N mineralization was water limited in acid sandplain lowland fynbos. Although it is impossible to determine which site characteristic is mechanistically responsible for higher mineralization rates, it is suggested that abiotic (moisture effects on soil microbes) rather than biotic (species effects on litter quality) differences are driving N cycling patterns in uninvaded fynbos. The lack of correlation between moisture and mineralization (Table 3.4.2) is probably due to the

low number of replicates ($n = 12$) and the low amount of variability within the mineralization data, even when compared on a monthly, as opposed to annual, basis (Fig 3.3.2).

In the comparison of sites 2 and 3 to test for the effects of stand age, soil organic matter and total N were not significantly higher in the older stands. This was surprising since the pools would have had longer to accumulate. Cocks and Stock (2000), however, also found a lack of consistent trends in soil total N or organic matter with stand age. Similarly, there were no significant differences between total N from soil under *L. parile* canopies and open spaces. This suggests that the fertile islands seen in other arid and mesic shrublands (e.g., Callaway 1995, Vinton and Burke 1995, Stock *et al.* 1999) do not exist here, a result corroborated by Stock and Lewis (1986), who found no significant lateral variation in soil total N. This lack of soil organic N despite larger litter inputs underneath canopies is probably most directly related to the slow rates of decomposition and the effect of regular fires (5-20 y) dispersing nutrients across the landscape. Indeed, it has been postulated that because of the extremely slow decomposition of litter, fires become the main source of nutrient release in the fynbos (Mitchell *et al.* 1986, Stock and Lewis 1986).

NH_4^+ mineralization rates, however, were higher in the older stands. This finding was contrary to that of Stock *et al.* (1988), who did not find an increase in N mineralization (NH_4^+ or NO_3^-) with age in stands of 1, 6, and 20 y old *Protea repens*. The only variables within this data set that could explain the increase in NH_4^+ mineralization are litter N and P, both of which were higher in the older stands (Table 3.5.2). The differences in litter quality could stem from changes in the proportions of leaves and flowers in the litter with age (Table 3.5.3). However, because the younger stand had a much higher percentage of flowers in its litter, which should have a higher N content than leaves, it seems unlikely that higher leaf N in older stands was due to changes in litter fractions through time. The difference in leaf N could also be a result of differential ages of the litter collected. Although every attempt was

made to find leaves that had recently fallen for the nutrient analyses, the leaves under the canopies of older *L. parile* had already turned brown. Because N and P are sequestered in *L. parile* litter for the first 18 months of decomposition (Witkowski 1991), immobilization of nutrients, and not stand age, probably explains the pattern of higher tissue nutrients in older stands. The increase in NH_4^+ mineralization, then, can not be explained by differences in leaf quality and may be due to other unmeasured biotic or abiotic factors. Stock and Lewis (1986) found high lateral heterogeneity in N mineralization rates, which they attributed to small variations in soil microclimate and N-use patterns of different species. A larger range of stand ages with higher replication would be useful in exploring the trend in N transformation rates during fynbos succession.

Vitousek and Walker (1989) claimed that one reason the N_2 -fixing invasive alien plant *Myrica faya* was able to cause large shifts in nitrogen cycles in Hawaii was the lack of native N_2 -fixers in early successional Hawaiian rainforest. Fynbos, on the other hand, has 644 species of Fabaceae (Bond and Goldblatt 1984). These legumes generally tend to only be prevalent in communities existing directly after fire (Kruger 1979). In acid sandplain lowland fynbos specifically, Fabaceae constitute 3-8 percent cover in the first 5 years after fire and decline to 1-2 percent cover afterwards (Hoffman *et al.* 1987). Why do native N_2 -fixers, then, not increase soil N status in pristine fynbos communities? Cocks and Stock (2000) investigated various *Aspalathus* spp. across several fynbos communities in order to ascertain the contributions of native legumes to the N budget in fynbos. Although *Aspalathus* spp. were most prevalent in early post-fire succession, and fixed between 30 and 100% of their own nitrogen, the N status of soils did not increase with stand age. They hypothesized that the lack of N accumulation was due to the heterogeneous distribution and ephemeral nature of native legumes in the post fire community -- the plants were not in one spot long enough to have any discernable effect on the soil. Indeed, low density, recent invasions of

N₂-fixing invasive aliens have little effect on the N status of soils in the fynbos (Witkowski 1991) and in Hawaii (Vitousek and Walker 1989), so it is of little surprise that native plants show the same pattern. Why native N₂-fixers do not have the same success in the fynbos as alien invasive legumes is still unknown (Cocks and Stock 2000), but can probably be explained by a lack of native herbivores for invasives and phylogenetic constraints on life history patterns of natives. Especially important would be constraints on native plant life span and persistence, architecture, and seed set (Richardson and Cowling 1992).

By investigating the effects of site on net N mineralization in the fynbos, it seems that soil moisture plays an important role in N cycling. Stand age had equivocal effects on N accumulation and mineralization and deserves further attention before patterns can be elucidated. Site effects were not looked at in other treatments within this study (grass, lupin, and acacia) because species and age differences were not apparent between sites. Furthermore, although site effects did exist for various soil nutrient pools, litter characteristics, and N mineralization rates, there was only one example (soil pH) of a site effect being significant without the treatment effect also being significant (Table 3.5.1). Overall treatment effects, therefore, were concentrated on for the remainder of the data set so that the effects on N₂-fixers would become apparent.

4.2 Fynbos vs. Acacia:

The ability to fix nitrogen, upon which *Acacia saligna* depends for 51% of its N budget (Stock *et al.* 1995), led to significant changes in ecosystem properties of the fynbos. The higher litterfall rates underneath acacia canopies, coupled with higher tissue N concentrations, resulted in more N being returned from the aboveground biomass to the soil (Fig 3.2.1 a, c, d). High inputs of litter N were the source of larger pools of soil organic

matter and total N below acacia canopies (Fig 3.4.1 a, d). Stock *et al.* (1995) substantiated the link between acacia N inputs and pools of soil organic matter and nitrogen with stable isotopes: soil became depleted in natural abundance $\delta^{15}\text{N}$ in acacia stands due to the negative $\delta^{15}\text{N}$ signature of the acacia litter. The higher concentrations of Ca^{++} , Mg^{++} , and K^{+} in acacia plots were probably due to the increased sequestration from the soil that couples higher primary production, and, consequently, litterfall under acacia invasion. Similar results of higher soil organic matter, cations, and total N have been documented in sandplain fynbos under *A. saligna* (Musil and Midgley 1990, Witkowski 1991, Musil 1993, and Stock *et al.* 1995) and in mountain fynbos and strandveld communities under both *A. saligna* and *A. cyclops* (Engledow 1989 and Stock *et al.* 1995).

Increased levels of organic matter and soil total N under acacia stands led to consistently higher mineralization rates of net NH_4^{+} and NO_3^{-} (Fig 3.3.2). Soil water availability, which was consistently higher in acacia stands (Fig 3.1.2 a), probably enhanced this effect by stimulating microbial communities (Alexander 1977). The importance of soil water and N inputs on net N mineralization rates can be seen in the multiple regression analysis. These results show that NH_4^{+} and total net N mineralization were dependent upon soil water and net NH_4^{+} mineralization was dependent upon both variables (Table 3.4.2 a, c).

While fynbos mineralization rates remained constant and low throughout the year, acacia soils experienced pulses which matched the first rains of the winter season, although both treatments had higher soil moisture contents at this time than during the summer (Fig 3.1.1 c and 3.1.2 a). The pulse effects are probably due to a mixture of moisture stimulating mineralization of the high soil organic N pool by microbes and the dependence of resin bags on water for transferring ions from the soil to the resin (Lajtha 1988). The dependence of resin bags on water may also be responsible for NO_3^{-} mineralization not being detected in the dry summer months, although this is probably also due to the high leaching rates of NO_3^{-} .

between high production periods since NH_4^+ was still detected by resin bags through the summer.

Acacia litter had lower P than that of fynbos, (Fig 3.2.1 b) implying that acacias are phosphorus limited -- typical of N_2 -fixing plants in low nutrient systems (Vitousek and Field 1999). *A. saligna* conserves its phosphorus, reabsorbing 71% of its leaf P before senescence, as opposed to the 48% reabsorption in *Leucospermum parile* (Witkowski 1991). This high phosphorus use efficiency in acacias constitutes further evidence of P limitation of the species (Vitousek 1982), which may explain the appearance of soil phosphorus as a dependant variable in the multiple regression analysis for NH_4^+ mineralization. Acacia stands released from P limitation, as could occur in invasions of agricultural fields that had been fertilized, may be able to increase N mineralization rates even further.

There was, however, a non-significant trend towards higher P in the acacia soils (Fig 3.4.1 e). Higher soil P accumulation could be explained by the calculation of P return to the soil (litterfall x litter P), which was higher in acacia than fynbos stands (15,024 and 6960 $\text{g m}^{-2} \text{y}^{-1}$, respectively). Engledow (1989) and Witkowski (1991) saw similar trends towards P accumulation under *A. saligna*. As seen with the increased levels of cations in acacia soils, the higher aboveground biomass and litter that accumulates in acacia stands (Milton 1981, Milton and Siegfried 1981) may effect other soil nutrient pools besides N.

The mineralization results from this study conflicts with those of Stock *et al.* (1995) who found no difference between mineralization rates under stands of *L. parile* and *A. saligna* in acid sand lowland fynbos, although acacia stands from both studies had existed for at least 15 years. The discrepancy is probably due to the different techniques used for estimating net mineralization rates: Stock *et al.* (1995) used the buried core method, which does not allow for the fluctuations in soil moisture that often lead to pulses in mineralization (Campbell *et al.* 1971). Therefore, data from this study refute the hypotheses suggested by

Stock *et al.* (1995) to explain the lack of change in net N mineralization with *A. saligna* invasions in the fynbos. These hypotheses include: (i) the fynbos not having the decomposer and microbial communities to handle increased litter inputs, (ii) *A. saligna* producing some secondary compound to inhibit decomposition that *A. cyclops* does not, and (iii) abiotic factors inhibiting mineralization in fynbos but not standveld. It is now clear that fynbos soil microbial communities are able to make use of increased inputs of organic N, leading to higher levels of N mineralization. The abiotic soil environment of fynbos, although not optimal for N mineralization processes due to lack of moisture, does not altogether inhibit the transformation of organic N into NH_4^+ and NO_3^- . Finally, although *A. saligna* probably produces secondary compounds that slow rates of decomposition (see Section 4.4) these are not strong enough to completely inhibit the N mineralization process.

4.3 Fynbos vs. Old-fields and Lupin:

Fields that had been plowed at least 32 years ago and left fallow for at least 15 years were not re-invaded by fynbos vegetation, but were covered in an herbaceous/grassy sward that may or may not have included dense stands of the annual lupin, *Lupinus luteus*. The high phosphorus concentrations in the old-field soils (grass and lupin), as compared to fynbos soils (Fig 3.4.1), suggest that a NPK fertilizer was added during cultivation. Because of this, fynbos data was included in all comparisons of N_2 -fixer vs. non N_2 -fixer in old-fields to establish a non-amended baseline for soil properties.

The plowing and fertilizing that had taken place in the old-fields increased rates of N cycling in the sandy soil, and these were further enhanced by the addition of an N_2 -fixing species. The fact that the old-fields maintained higher rates of N cycling after 15 years of abandonment implies that a positive feedback cycle had established (Vitousek 1982, Hobbie 1992). It is hypothesized that fertilization increased levels of inorganic N causing plants to

grow faster and with increased tissue nitrogen. The litter, with its lower C:N ratios, stimulated decomposition and N mineralization, keeping levels of inorganic plant available N high. Similar positive feedback cycles have been maintained in fertilized fields in Colorado, which still exhibited higher levels of N cycling after 20 years (Vinton and Burke 1995).

Data from this study support the above positive feedback hypothesis. The grass treatment had higher tissue N, litterfall, and N return to the soil, which resulted in higher annual net N mineralization rates than in the fynbos (Fig 3.2.1 and 3.3.3). Lupin, probably due to its ability to fix nitrogen, had higher values for tissue N, litterfall, and N return to the soil than the grass treatment (Fig 3.2.1). Lupin litter decomposed much faster than any other litter type in the study (Fig 3.7.1), probably because it had a C:N ratio of about 30 (see Section 4.4). Even though more biomass and N were being added to the soil annually in lupin as compared to grass treatments, the quick decomposition and lack of immobilization in lupin litter led to smaller pools of soil organic matter and an equivalent amount of soil total N (Fig 3.4.2). The high rate of N cycling under lupin is apparent in net N mineralization rates (Fig 3.3.3), which were higher in lupin treatments than in grass or fynbos. The higher N availability in lupin stands also resulted in higher tissue N concentrations for *Cynodon dactylon*, a grass growing in both treatments, under lupin as opposed to in the grass treatment.

The depletion of phosphorus under lupin as compared to grass suggests that the increase in biomass that compliments the ability to fix nitrogen in this species is draining P reserves in the soil (Fig 3.4.2 e). Furthermore, the inclusion of soil P as a dependant variable affecting net NO_3^- mineralization suggests that lupin, as with acacia (Section 4.2), may be P limited in this system (Table 3.4.2 b).

As in the acacia treatments (Section 4.2), soil water played an important role in determining rates of net N mineralization in the grass and lupin treatments. Soil moisture was greater in the grass treatment than any other (Fig 3.1.2 b) – this was due to the soils

becoming water logged (personal observation) during the late winter months when rainfall was highest (Fig 3.1.1 c). Nitrification was, therefore, probably inhibited in these plots due to the dependency of nitrifying organisms on oxygen (Alexander 1977), leaving available nitrogen in the form of NH_4^+ during late winter. The lack of difference between grass and fynbos treatments in net NO_3^- mineralization, then, would have been due to the absence of nitrification in the grass treatment because of waterlogging, a result also found in saturated sedge soils in Alaska (Nadelhoffer *et al.* 1991). NH_4^+ pooling in the grass treatment probably led to the increases in NH_4^+ mineralization relative to both fynbos and lupin during the later winter months (August, September, and October), which corresponded with the highest soil moisture and rainfalls. The lupin treatment maintained a higher annual net NH_4^+ mineralization rate than grass, however, due to its pulse in NH_4^+ mineralization with the first rains of winter (Figs 3.3.2, 3.1.2 b and 3.1.1 c). Indeed, the multiple regression model showed that soil moisture was an important factor determining NH_4^+ and NO_3^- and total net N mineralization rates in the grass treatment (Table 3.4.2 a, b, see also Section 3.4).

4.4 Comparing N_2 -fixing Invasives:

Biological invasions present a unique opportunity to study the impact of a species on a community or ecosystem, giving insight into how those systems function. The impact of the invasive species is thought to be the greatest when it represents a new guild or functional group, as in the case of N_2 -fixing alien invasives (Vitousek and Walker 1989). Normally, studies of species level effects on ecosystems concentrate on two species which belong to different guilds, such as N_2 -fixers and non N_2 -fixers (e.g., Vitousek and Walker 1989, Garcia-Montiel and Binkley 1998), or within annual, herbaceous, and grassy guilds (e.g., Wedin and Tilman 1990, Vinton and Burke 1995, Wardle *et al.* 1998, Christian and Wilson 1999). By

studying the impacts of two N₂-fixing invasives that belong to the same guild and within the same community type, however, it is possible to tease apart the mechanisms that lead to differential effects of N₂-fixing invasive plants on ecosystem functioning. With this information it may be possible to make predictions concerning the possible impacts of other N₂-fixing invasive species.

For this reason, the effects of lupin and acacia on N cycling in the fynbos were investigated further. Statistical comparisons between field data from acacia and lupin treatments were not made due to the history of fertilization in the lupin soils (Section 4.3), but obvious trends do exist between the two species. N return to the soil was higher in the lupin treatment, primarily due to larger amounts of litter rather than higher tissue N concentrations (Fig 3.2.1). Higher litterfall rates in lupin may have been due to a larger annual biomass accumulation or a release from P limitation due to past fertilization. Net NH₄⁺ mineralization rates were higher in lupin plots, although net nitrification rates were similar, leading to slightly higher total net N mineralization under lupin (Fig 3.3.2 and 3.3.3). Surprisingly, soil organic matter and total N were lower underneath lupin stands (Fig 3.4.1 a and 3.4.2 a). These results indicate that although more litter was falling in the lupin treatment, nitrogen was moving from the organic to available pools faster due to quicker decomposition rates (Fig 4.4.1).

Controlled microcosm experiments proved that lupin litter had higher initial rates of decomposition than acacia litter. The highest respiration rates, and therefore decomposition rates, were found for lupin litter (Fig 3.7.1 a, b). Generally, lower C:N ratios in leaf litter are indicative of faster decomposition rates, where the ratio ~ 30:1 determines the cut-off between mineralization and immobilization of nitrogen. If the C:N is greater than 30:1, N will be immobilized in the litter; if the C:N is below 30:1, then N mineralization will take place (Wedin 1999). Acacia litter proved to be the anomaly, having initial decomposition

rates equivalent to those for fynbos litter even though acacia leaf C:N ratios were as low as those for lupin litter (Fig 3.7.1 c). Regressions of soil respiration as a function of leaf N also showed acacia litter to have lower than expected initial decomposition rates. Within all of the treatments, litter N content explained only 46% of the variance in respiration rates, but when the acacia data was removed, leaf total N explained 76% of the variance (Fig 3.7.2). Previous studies, however, have shown that decomposition should occur faster in acacia than fynbos litter. *L. parile* litter, the same target species as in this study, immobilized nitrogen over a year of decomposition, whereas *A. saligna* litter N concentration did not change through time (Witkowski 1991) implying that microbial N limitation should not be slowing decomposition and N mineralization. Although *L. parile* has high lignin levels (Mitchell *et al.* 1986), no measurements of this kind have been made for *A. saligna* litter. Secondary carbon compounds, lignins, or polyphenols may slow litter decomposition even when microbes are not N limited (Hättenschwiler and Vitousek 2000), leading to the differential initial decomposition rates between the two N₂-fixers despite similarities in leaf tissue N.

A schematic representation of hypothesized N pools and transformations in established invasions of lupin and acacias (Fig 4.4.1) shows the important relative differences between the species. Most of the N in the acacia system is locked in the aboveground biomass. Of the acacia litter that falls, less N is decomposed and mineralized into plant available forms (NH₄⁺ and NO₃⁻) than in the lupin treatment. The perennial life histories of acacias, however, result in additional biomass and subsequent litterfall each year. Lupins, on the other hand, will have similar annual rates of aboveground biomass regrowth and litterfall (per m²). The additional acacia litter input, coupled with slower decomposition rates of litter, will result in a growing pool of soil organic N and rising mineralization rates through time (Fig 4.4.2). Lupin systems, however, will have unchanging annual net N mineralization rates that do not increase on a yearly basis. This static mineralization rate will be due to the

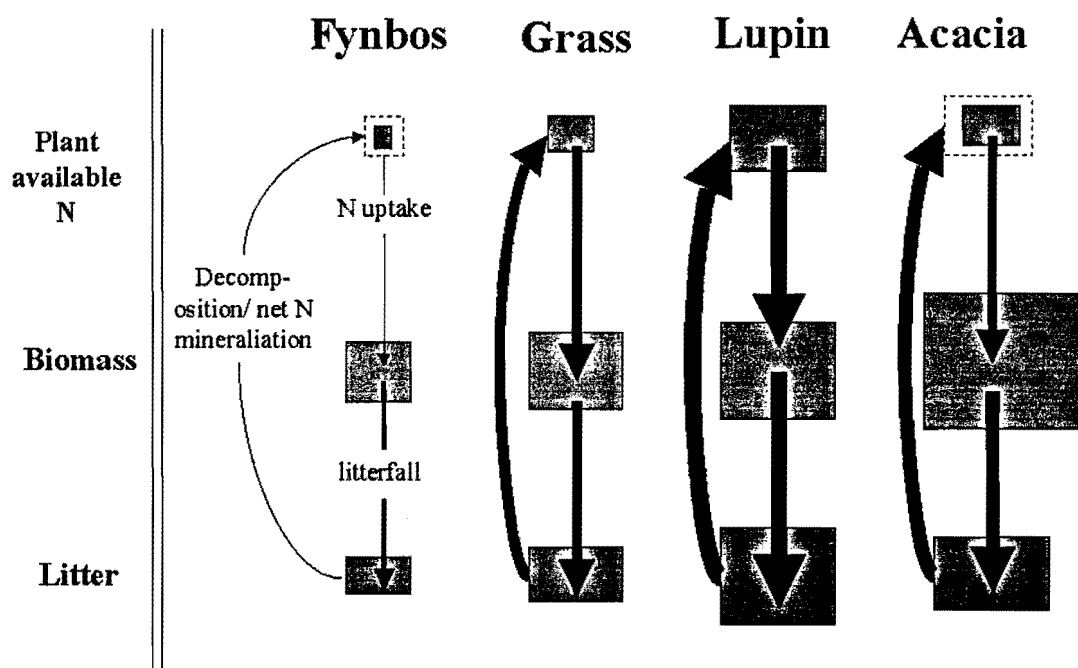


Figure 4.4.1. A schematic diagram representing the pools (boxes) and transformations (arrows) of N in fynbos, grass, lupin, and acacia systems. The size of the boxes and width of the arrows are based on relative differences in the sizes of N pools and N transformation rates, respectively. Stippled boxes represent increases in the size of the plant available N pool (NH_4^+ and NO_3^-) after one complete N cycle.

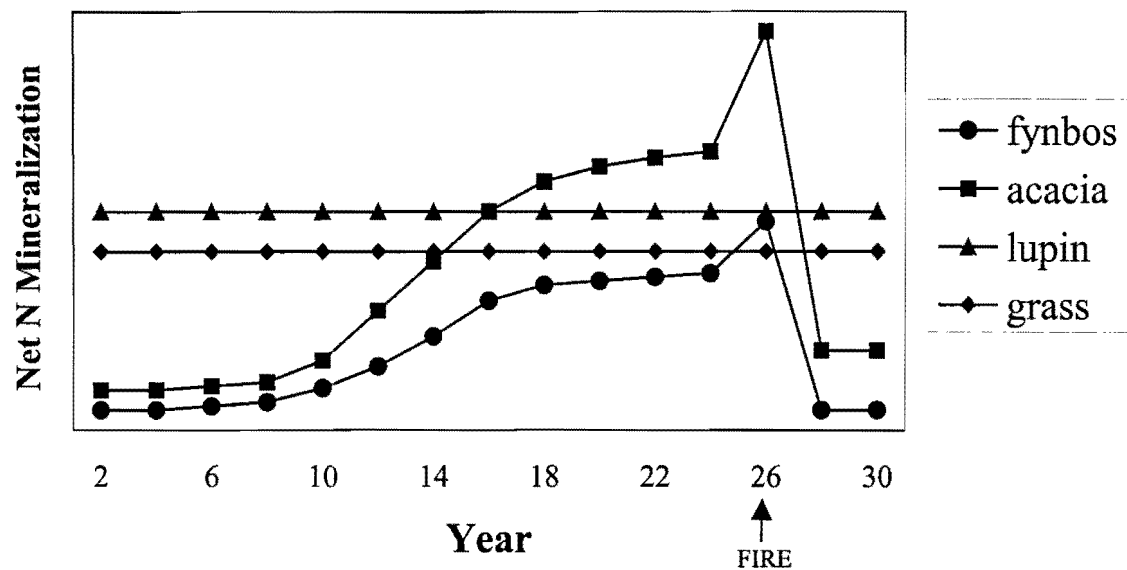


Figure 4.4.2. A theoretical model showing rates of net N mineralization through time in fynbos, acacia, lupin and grass systems. The model includes one fire after 26 years of stand growth.

steady-state balance of plant uptake, growth, litterfall, and microbial mineralization in the annual system. The grassy old-fields would function similarly, although at lower levels, to the lupin systems due to their annual nature. Therefore, plant available N would not show an annual increase through time in grassy systems. Fynbos plants, which are largely perennial like acacias, would accumulate organic N through time, albeit at a much slower rate. Fire (see Section 4.7), through volatilization of N, would bring the perennial systems back down to lower levels of organic N and net N mineralization. The length of time perennial fynbos and acacia systems have grown since fire, therefore, will determine their N cycling rates relative to each other and the annual lupin and grass systems.

Species level differences in life history traits and litter quality have been found to predict soil property changes within communities. In this study, differences in acacia and lupin litter quality, in terms of secondary compounds rather than tissue N concentration, were probably responsible for the decomposition and N mineralization rate differences between the different species. Similarly, studies investigating soil properties underneath different species of the same guild and habitat have found available nitrogen, (Wedin and Tilman 1990, Christian and Wilson 1999), soil respiration and biomass (Wardle *et al.* 1998), or both (Vinton and Burke 1995) to change with species. Wardle *et al.* (1998) correlated whole plant traits, as opposed to those for litter only, to rates of decomposition. They found that plants that were shorter lived, attained smaller sizes, and concentrated more nitrogen in their tissues decomposed faster. This list of plant traits also fits the differences between lupin and acacia due to the differences in perennial and annual life histories. Whether using plant or litter traits to make predictions, however, the changes in overall net N mineralization and the form of available nitrogen may feedback onto later community dynamics. Within fynbos native

species, there are differential preferences to NH_4^+ and NO_3^- . *Protea repens*, for example, uses NH_4^+ preferentially to NO_3^- (Stock and Lewis 1984).

Differential decomposition rates will also become important in terms of predicting how long elevated levels of soil nitrogen will last after alien species are cleared, given that the invasions had been established for similar lengths of time. Maron and Jefferies (1999) predicted that elevated levels of nitrogen in soils underneath *Lupinus arboreus* would take about 25 y to dissipate by half. Similar trends may be expected for the lupin species in this study, *Lupinus luteus*, but organic nitrogen originating from *A. saligna* will take longer to mobilize into plant available forms due to slower decomposition and mineralization rates. Therefore, there will probably be larger pools of available nitrogen, especially in the form of NO_3^- , under cleared lupin stands in the first season after restoration. Nutrient cycling which is enhanced in comparison to native fynbos, however, will persist longer under cleared acacia stands. It may be more difficult, then, to restore the native community within lupin invaded areas than acacia stands due to higher levels of available N resulting in a greater chance of secondary invasion by weedy grasses and forbs directly after clearing (see Section 4.5).

From a methodological standpoint, the lack of increased soil total N in lupin stands also demonstrates the importance of quantifying labile fractions of N as well as static pools. If rates of mineralization were not obtained, it might have been assumed that rates of N cycling under lupin were no higher than in the grass treatment. Vinton and Burke (1995) also found that species effects on soil geochemical cycling were most apparent in the labile fractions of C and N.

4.5 Ecosystem Feedbacks:

Plants not only affect the nutrient status of soils, but these changes feedback to affect population and community processes such as growth, competition, and succession (Hobbie 1992). In acid sand lowland fynbos, the densely packed invasions of various alien annual and native weedy perennial grasses (in particular, *Ehrharta calycina*) in cleared acacia stands (personal observation) are probably due to increased nutrients in the soil, the lack of a native seed bank, and the high seed availability of invasives. Because the land where these observations were made had been previously plowed, and possibly fertilized, prior to acacia invasion, there is still speculation as to whether unaided invasions will have the same effect. Holmes and Cowling (1997 b), however, noted the same pattern of high percentage cover of grassy and annual herbaceous vegetation after clearing unaided invasions in lowland fynbos. The high biomass in these areas is counter to the successional model for acid sand lowland fynbos (Hoffman *et al.* 1987). In this model, the dominant species in years 1-3 after fire were ericoid-leaved shrubs; annuals were not abundant and *E. calycina* was not noted at all. The percent cover of natural vegetation after fire was less than 30% after one year, less than 40% after two years, and only reached 58% cover by three years. High vegetation cover due to increases in weedy species in the first years after fire may create a system in which native species have a difficult time competing for light and other resources.

Data from this study support the hypothesis that increased N in soils from *A. saligna* invasions will facilitate secondary invasions. The higher N status of acacia soils resulted in higher growth rates of *E. calycina*. The higher biomass and lower root:shoot ratios of *E. calycina* in these soils indicate a release from nutrient limitation (Fig 3.6.1 a, c). The grass did not increase tissue N concentrations in enriched soils, instead using the excess N to create more biomass, leading to greater shoot N content per plant (Fig 3.6.1 b, d). *E. calycina* exhibited a logistic growth curve (Fig 3.6.2 a), which flattened to give a somewhat constant

biomass yield at about 0.7 mg N g soil⁻¹, well above the maximum amount of total N (0.25 mg N g soil⁻¹) found in any of the Riverlands fynbos sites. This suggests N limited growth of *E. calycina* on fynbos soil and helps to explain its lack of abundance in the biome (Hoffman 1987) even in fynbos patches close to *E. calycina* seed sources at reserve boundaries (personal observation). *E. calycina*, therefore, may have a competitive advantage over slow growing fynbos seedlings in enriched soils, changing overall community dynamics after acacia invasions have been cleared.

Why would higher nutrient levels in the soil lead to changes in community structure? For all types of fynbos, post-fire succession involves the vast majority of the seeds germinating at one time. There is little regeneration of species between fires and succession is defined by each species' life history -- the dominant species will be those that have achieved the greatest biomass by that time, and species drop out of the sequence based on life span (Bond and van Wilgen 1996). Therefore, the growth rate of each species is important in terms of community dominance. Competition for specific resources, especially nitrogen, is not thought to explain species distributions in the fynbos (Bond *et al.* 1992). Fynbos plants are pre-adapted to low nutrient regimes, having slow growth rates and little plasticity in these rates with changing resource levels (Stock *et al.* 1990). Ruderal species such as *E. calycina*, however, are able to increase growth rates, and therefore, aboveground biomass, in the presence of increased soil resources (Grime 1977). Within the context of post-fire succession, therefore, fast growing ruderal species that gain the biomass advantage early in the growing season may quickly overtop native fynbos species, which are generally thought to be shade intolerant (Cowling and Gxaba 1991). This proposed pattern will be aided by the winter germination life histories of grasses such as *E. calycina*, *Briza maxima*, *Avena fatua*, *Lolium perenne*, and *Cynodon dactylon* and the concomitant winter pulse in N mineralization which occurs in acid sandplain lowland fynbos soils after invasions by N₂-fixing plants (Figs

3.3.2 and 3.3.3). Dominance of the post restoration community by grasses may also increase fire cycles (D'Antonio and Vitousek 1992), especially in the fire prone region of Riverlands Nature Reserve (Kilian 1995), further suppressing native shrubby vegetation by killing individuals before reproductive maturity.

E. calycina is not the only grass to exhibit increased growth in nutrient enriched fynbos soils. In a similar experiment, the invasive alien grass *Bromus diandrus* achieved greater biomass in soils from acacia invasions than nearby uninvaded acid sandplain lowland fynbos (Simons 1999). Grass invasion and soil enrichment may also be linked in urban acid sandplain fynbos fragments, where N deposition from airborne pollutants seems to be enhancing the invasion of grasses (Jobst 1996, Wilson 1999).

In contrast to grasses, sclerophyllous fynbos shrubs tend to show low plasticity in growth patterns when encountering excess nutrients in the soil, keeping growth rates slow, and storing the nutrients for later use when soil stores are depleted (Witkowski *et al.* 1990 b). The proteoid shrubs *Protea repens* and *Leucospermum parile*, as well as the ericoid shrub *Phyllica cephalantha* all have minimal growth rate responses to nutrient additions (Stock and Lewis 1984, Witkowski *et al.* 1990 b). Some studies show that excess nutrients can even have a negative impact on fynbos species. For *P. repens*, N:P imbalances in potting soil decreased growth rates and caused mortality in seedlings (Witkowski 1989 a) while nutrients added in the field decreased growth rates of mature individuals (Lamb and Klaussner 1988). Plants adapted to low nutrients also tend to have slower rates of nutrient acquisition (Chapin 1980), as seen in *P. repens*, whose assimilation rates of nitrate and ammonium are considerably slower than fast growing grasses (Stock and Lewis 1984).

The invasion of grasses after clearing may also help to keep increased levels of nitrogen cycling high via a positive feedback cycle of yearly inputs of N rich litter which then continue to stimulate microbial activity and N mineralization (Vitousek 1982). *E. calycina*

growing on acacia N rich soil increased its biomass (Fig 3.6.1 d) while maintaining C:N ratios at about 15.7 (± 3.2 , $n = 24$). These C:N values indicate that large amounts of biomass will be returned to the soil annually and will quickly decompose and become mineralized to plant available N (Wedin 1999). Tateno and Chapin (1997) maintain that N_2 -fixing invasives will not lead to long term changes in N pools. Their model suggests that a negative feedback cycle will be caused by the plants recolonizing areas having either fast growth rates and high C:N ratios or low growth rates and low C:N ratios. These combinations would cause a convergence in litter quality and therefore would slow N cycling. It is demonstrated here, however, that recolonizing plants can have high relative growth rates and low C:N ratios, thereby maintaining ecosystem shifts in the long term.

Secondary invasions will also be aided by changes in seed bank dynamics. According to Hobbs and Atkins (1988), invasions require the successful dispersal and persistence of the propagules of the invasive plant species. Seed of native and non-native invasive grasses such as *E. calycina*, *Briza maxima*, *Avena fatua*, *Lolium perenne*, and *Cynodon dactylon* are obviously available at Riverlands since these species exist within the confines of the reserve (Kilian 1995) and also exist within the larger context of the fynbos (Linder 1989). *E. calycina* and *C. dactylon*, although native, are considered invasive in this context because they have not been found to play significant roles in terms of biomass or individual numbers in early or late acid sand lowland fynbos succession (Hoffman *et al.* 1987). Because the reserve is bounded on all sides by agricultural land, it is quite probable that seed is dispersing in from outside, as was found in Australian reserves by Hobbs and Atkins (1988). Native seed, on the other hand, is not readily available. Studies in fynbos across the Cape Peninsula have shown that after *A. saligna* invasions of 25 years, regeneration of native species is lower for most guilds and absent in some of the large reseeders such as the Proteaceae due to the loss of the seed bank (Holmes and Cowling 1997 b). Fynbos seed in general has very short

dispersal distances, so it may not be able to recolonize land after invasions have been cleared (Holmes and Richardson 1999). After clearing, therefore, native seed densities will be low and weedy seed densities will be high, enhancing the competitive effects caused by enriched soils.

Alien plant invasion after nutrient enrichment has been noted in other systems besides the fynbos. The invasive bush lupin, *Lupinus arboreus*, has been shown to increase soil nitrogen levels, leading to secondary invasions of alien invasive grasses such as *B. diandrus* in Californian dunes (Maron and Connors 1996, Pickart *et al.* 1998 a). In the Mojave Desert, species richness and abundance of alien annuals was greater on soils of higher fertility (Brooks 2000). The spread of invasive alien nitrophilic plants is often attributed to N enrichment through atmospheric deposition (e.g., Bobbink *et al.* 1998, Padgett and Allen 1999) while the invasion of European grasses in Californian shrubland has been correlated with higher rates of nitrogen cycling on highly disturbed sites (Zink *et al.* 1995). Experimental studies have shown a similar pattern. Alien plant abundance increased with fertilization in the Australian heathlands (Specht 1963), the Australian wheatbelt, (Hobbs and Atkins 1988), the heathlands of Netherlands (Berendse *et al.* 1987), Californian serpentine grasslands (Hobbs *et al.* 1988, Huenneke *et al.* 1990), the shortgrass steppe of Colorado (Vinton and Burke 1995), and British limestone grasslands (Burke and Grime 1996). Evidence from other systems, then, lends support to the hypothesis that secondary invasions of weedy species will occur in the fynbos after the clearing of N₂-fixing invasive alien plants.

4.6 Comparisons to Other Systems:

In the course of succession within natural systems, N₂-fixing species are generally thought to facilitate later species by increasing soil nitrogen levels (Connell and Slatyer

1977). In agriculture, N₂-fixing annuals are used as "green manure" to enrich soils between crops of grain. It should come as no surprise, therefore, that invasive alien N₂-fixing species also enrich soils. Although there are 49 species of invasive plants from the Fabaceae (Daehler 1998), the largest group of potential nitrogen fixers, the ecosystem impacts of only five N₂-fixing species have been investigated. All five of these species, however, have quantitatively been shown to increase rates of N cycling in the communities they invade. These include *Myrica faya* in Hawaiian montane tropical forests (Vitousek et al. 1987, Vitousek and Walker 1989), *Lupinus arboreus* in coastal Californian sand dunes (Maron and Connors 1996, Maron and Jefferies 1999, Pickart et al. 1998 a), *Acacia cyclops* in South African standveld (Stock et al. 1995), and now *Acacia saligna* and *Lupinus luteus* in South African fynbos. Vitousek and Walker (1989) claimed that three requirements must be met before an invasive species can be said to significantly alter N cycling: (i) nitrogen must be limiting in the pre-invasion system, (ii) the invasive alien must change inputs of nitrogen, and (iii) the nitrogen inputs must become available to other organisms. It is proposed here that a fourth point should be added: (iv) the excess available nitrogen in the system should be detrimental to native flora. This final point suggests that the N cycling shift should have an effect on subsequent community dynamics to be considered significant. These criteria may then be used to ask what these particular invasives and invaded communities have in common.

Fertilization experiments proved that native species in the Hawaiian and Californian systems were nitrogen limited (Vitousek and Walker 1989). However, nitrogen limitation in the classical sense -- native plants exhibiting increased biomass in response to fertilizer -- may not be the best indicator of the susceptibility of communities to ecosystem shifts caused by N₂-fixing invasives. Native species in low N systems are usually adapted to storing, rather than assimilating nutrient pulses into biomass. Chapin et al. (1986) predicted that because of

this, low nutrient sites have a large potential to increase in productivity, not through increased growth rates of the native plants that are adapted to living in those sites, but through changes in species composition to plants that show greater plasticity in growth rates. Many fynbos plants, especially the sclerophyllous shrubs, respond negatively or not at all to fertilization treatments (Lamb and Klaussner 1988, Witkowski 1989 a, b, Witkowski 1990 b), although alien or native weedy grasses respond well, as in the coastal California sand dunes (Maron and Connors 1996). Fertilization experiments should not be ruled out when testing for the N limitation of ecosystems, but a negative or non-response to fertilization should not be used to suggest that a community is not susceptible to N cycling changes after invasions of N_2 -fixers. A low overall level of N cycling may also be a good indicator of N limitation and susceptibility to elevated N within a community.

Nitrogen inputs, as shown by soil nitrogen pools, were increased by all five species investigated due to increased litterfall and tissue N concentrations (see Table 4.6.1 for references). Decomposition rates were higher for the litter of invasive species, leading to higher levels of available nitrogen in the soils underneath all invasives. Higher available nitrogen fulfilled the last caveat of Vitousek and Walker (1989) for an invasive to significantly change ecosystem properties. The simple equation of high N litter into a low N system, however, is complicated by time: soil N content increased with length or density of invasion in all three species for which these variables were tested (Table 4.6.1). It seems, then, that there is a threshold of time and/or density of invasion that must be surpassed before the ecosystem process changes.

Finally, increased available nitrogen was shown to be detrimental both directly and indirectly to native communities in California and South Africa. Directly, nitrogen can be toxic to native species, as was found for *P. repens* in the fynbos (Lamb and Klaussner 1988, Witkowski 1989 a). Indirectly, nitrogen can increase the chances of a secondary invasion by

Table 4.6.1. Properties of different systems and their respective N₂-fixing invasive aliens (in comparison with native vegetation). N limitation of site is in reference to fertilization studies on native vegetation. Leaf decomposition was determined by mass and nutrient lost in *M. faya*, *A. cyclops*, and *A. saligna* and by microbial respiration rates in *L. luteus*. Available N is defined as net mineralization rates of NH₄⁺ and NO₃⁻ for all species except *M. faya*, which was determined as potential net mineralization of NH₄⁺ and NO₃⁻. Referances: (1) Vitousek and Walker 1989, (2) Maron and Jefferies 1999, (3) Pickart *et al.* 1998a, (4) Maron and Connors 1996, (5) Witkowski 1989b, (6) this manuscript, (7), Witkowski 1989a, (8) Lamb and Klausner 1988 (9) Milton 1981, (10) Witkowski 1991, (11) Stock *et al.* 1995, (12) Musil 1993, and (13) Musil and Midgley 1990.

Species	Location	Initial N limitation of site	Higher litterfall	Higher tissue N	Higher total soil N	Invasion length/density effect	Faster leaf decomposition	Higher available N	N cycling shift detrimental to native flora
<i>Myrica faya</i>	Hawaii	Yes ¹	Yes ¹	Yes ¹	Yes ¹	Yes ¹	Yes ¹	Yes ¹	N/A
<i>Lupinus arboreus</i>	California	Yes ²	N/A	N/A	Yes ²	Yes ³	N/A	Yes ^{2,3,4}	Yes ⁴
<i>Lupinus luteus</i>	South Africa	No ⁵	Yes ⁶	Yes ⁶	Yes ⁶	N/A	Yes ⁶	Yes ⁶	Yes ^{6,7,8}
<i>Acacia cyclops</i>	South Africa	No ⁵	Yes ^{9,10}	Yes ¹⁰	Yes ^{10,11}	N/A	Yes ¹⁰	Yes ¹¹	Yes ^{6,7,8}
<i>Acacia saligna</i>	South Africa	No ⁵	Yes ^{6,9,10}	Yes ^{6,10}	Yes ^{6,10,11,12,13}	Yes ¹⁰	Yes ⁶	Yes ⁶	Yes ^{6,7,8}

weedy species, which then may exclude native species from recolonizing. Secondary invasions due to ecosystem shifts were well demonstrated by Maron and Connors (1996). They showed a concomitant increase in available N and plant biomass and a decrease in native species and overall species richness underneath dead patches of *L. arboreus*. A glasshouse bioassay was also used to show that invasive alien grasses obtained more biomass in soils from underneath dead *L. arboreus*. This study showed that secondary invasions by the weedy species *E. calycina* would be more likely after acacia invasions because the grass obtains higher biomass in soils from underneath *A. saligna* stands.

Interestingly, in the mediterranean systems of California and South Africa, the only two in which monthly mineralization rates have been measured underneath N₂-fixing invasives, large pulses of available nitrogen with the onset of winter rains were found (Fig 3.3.2 and 3.3.3, Stock *et al.* 1995, Maron and Jefferies 1999). These pulses, which did not exist in the native communities, match the winter growing season of weedy and invasive grasses. Therefore, system switches might not only be important in terms of overall levels of available nitrogen, but the timing of release as well.

Generalizations about the effects of N₂-fixing invasives on N cycles can be made given the information in the above comparisons. All five species investigated increased inputs of N into the soil through higher litterfall rates in combination with N rich litter. These inputs led to higher levels of organic and available N in all of the systems. The higher decomposition and mineralization rates for all invasive N₂-fixing species indicate that, even in low N systems, the production of available N was never limited by microbial communities or abiotic factors. Changes in N cycling, however, seem to be positively correlated to the length of invasion. Therefore, it seems probable that any invasive which is actively fixing nitrogen in an ecosystem that has relatively low pre-invasion levels of N cycling will cause ecosystem shifts, given large enough densities and/or enough time. How long it will take for

N cycling shifts to take place will be dependent on quantitative differences between native and invasive vegetation for factors that affect N inputs. These factors include those that directly determine N quantity being returned to the soil such as litterfall rate and litter N concentrations. Also included, however, are those factors that indirectly affect decomposition and mineralization processes such as litter secondary carbon compounds and changes in soil microclimate.

4.7 Effects of Acacia Clearing and Fire on N Cycles:

It was predicted that the clearing of acacia stands would elevate N mineralization rates in the soil due to changes in soil microclimate. This would mean that restoration efforts might actually intensify the enhanced N cycling effect of N₂-fixing invasive species. Indeed, soil temperatures fluctuated more in cleared stands, becoming hotter when the sun shone directly on the soil and colder during the night or on cold, overcast days. On overcast days the temperature was higher in the closed stands due to heat conservation from plant transpiration.

Despite overall statistical differences in temperature (Fig 3.1.2 c), however, mineralization rates did not differ between cleared and uncleared treatments (Fig 3.3.2). Optimum temperatures for NH₄⁺ mineralization are between 40°C and 60°C (Alexander 1977), and these levels were never reached, even in cleared stands. Optimum temperatures for NO₃⁻ mineralization, which are between 30°C and 35°C (Alexander 1977), were only achieved on the March sample date in cleared stands. Temperature effects on soil processes have been shown at much lower levels, however, in arctic soils. Nadelhoffer *et al.* (1991) found that N mineralization increased 10 fold when temperatures were changed from 9°C to 15°C in controlled soil incubation studies. From this study it does not seem as if N

mineralization rates will be affected by the increases in soil temperatures from the clear cutting of acacia stands.

The lack of change in N mineralization rates may also be due to the reduction of soil moisture in the cleared acacia stands. As stated above (Section 4.2), N mineralization in acacia stands is, at least in part, dependant on soil moisture. Elevations in temperature, then, would indirectly inhibit microbial transformations of nitrogen due to evaporation of water from the soil. It is unclear which would have the overriding effect on N mineralization, the negative effects of decreasing soil moisture or the positive effects of increased temperatures. Controlled incubations of soils with a fully factorial set of temperature and moisture regimes would be needed to answer this question.

The effects of clearing vegetation on N mineralization rates have generally been investigated in terms of fire due to the removal of aboveground biomass. Blair (1997) proposed two hypotheses to explain increases in aboveground net primary production (ANPP) in grasslands after infrequent fires. The “enhanced N mineralization hypothesis” predicted that accumulated soil organic matter coupled with higher soil temperatures after the removal of standing biomass would cause a pulse in N mineralization after fire, leading to higher aboveground net primary production. The “transient maxima hypothesis” predicted that increases in aboveground biomass were due to the greater relative availabilities of both light and soil nitrogen directly following fires. Because N mineralization rates under *A. saligna* stands in the fynbos are not limited by light, neither hypothesis would explain N mineralization patterns in the fynbos.

Even though it is not due to an increase in light and soil temperature, fires still have important effects on N cycling in the fynbos. Stock and Lewis (1986) found exchangeable NH_4^+ and NO_3^- levels to increase in fynbos acid sandplain soils in the 4-9 months directly following a fire. This pulse was not attributed to a release from light limitation, but to ash

deposition from the burnt aboveground biomass and the breakdown of organic matter in the upper layers of the soil. Similar effects of ash deposition were seen in pine forests in California (Grogan *et al.* 2000) although no effect was seen in Kansas grasslands (Hulbert 1988). The data from this study help tease apart the physiochemical direct effects of fire (breakdown of organic matter and ash creation) and the indirect effects on soil microclimate (changes in temperature and soil moisture). During early post-fire succession in the fynbos, therefore, it seems that fire plays a larger role in determining N mineralization rates than abiotic factors. The importance of biotic rather than abiotic factors has also been stressed in incubation studies investigating factors controlling rates of N mineralization; these have shown that the effects of differential soil organic matter quality are more important than the effects of temperature (Nadelhoffer *et al.* 1991). It is predicted, then, that the extra aboveground biomass and organic matter in the soil due to invasions will lead to larger available N flushes in invaded than uninvaded soils after cleared acacia stands are burned. Furthermore, these N mineralization flushes will not be significantly enhanced by changes in soil microclimate due to vegetation clearing.

Incorporating data from this study with existing knowledge concerning fire and N cycling in fynbos, it is possible to create a model that predicts how fire will further affect ecosystem shifts due to invasions of acacias. Although data concerning N mineralization patterns along successional gradients are equivocal, pools of total soil N were shown to increase along a chronosequence culminating in stands that were 20 y old (Stock *et al.* 1988). These increases in organic N material may lead to increasing rates of N mineralization through time (Fig 4.4.2). Volatilization of organic N in fire would then cause an overall loss of system N, resulting in a sharp decrease in N mineralization after post fire flushes subsided (Stock and Lewis 1986). A similar pattern may exist in long term acacia invasions, although in this system, rates of N mineralization would increase faster through time due to larger

inputs of N rich litter that decompose more quickly (see Section 4.2, Figure 4.4.1).

Therefore, post fire flushes of available N and N mineralization rates may also be higher in invaded systems. This can be predicted for two reasons: (1) larger amounts of standing litter will increase ash inputs and (2) larger pre-fire pools of organic matter will result in proportionately less soil organic N being volatilized in the fire. Therefore, after each fire cycle, acacia stands will already have a “head start” towards higher rates of N cycling. It is uncertain at what level the steady state net N mineralization rates would exist for the annual lupin and grassy old-fields within this model (Section 4.2). Because fire records for the specific sites within Riverlands Nature Reserve do not exist, we have no way knowing where each system sits relative to the others concerning length of time since the last fire.

Increasing the frequency of fire would change the above model from one in which N mineralization is limited by biological processes (i.e., microbial communities) into one in which N mineralization is limited by physical processes (i.e., fire). At short fire intervals, levels of soil organic N, and therefore mineralization rates, would not be given enough time to increase before burns (Fig 4.7.1). The only N mineralization to take place would be due to the combustion of aboveground biomass resulting in brief pulses of available N after fires. Under these conditions, the effects of acacias on N cycling would be less apparent. Once released from high fire frequencies, the perennial systems would start accumulating soil organic N once again.

4.8 Implications for Restoration:

The first step to take in restoring the native community is to ask how community and ecosystem functions have changed (Holmes and Richardson 1999). According to Richardson and Cowling (1992), plant invasions by trees or large shrubs change community function by

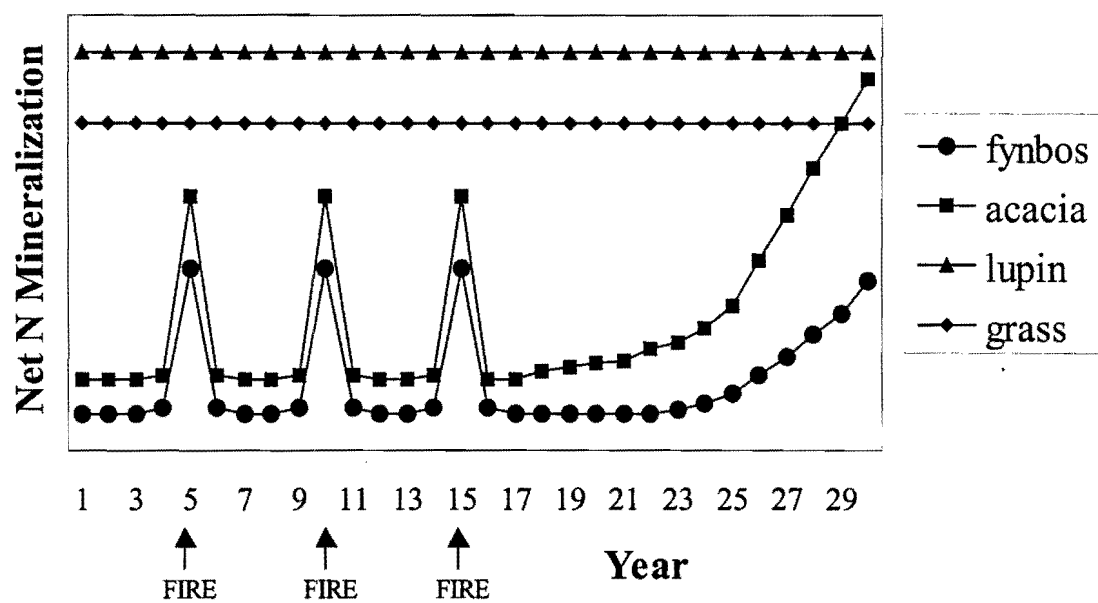


Figure 4.7.1. A theoretical model showing rates of net N mineralization through time in fynbos, acacia, lupin and grass systems. The model includes one fire every 5 years for three fire cycles and then a subsequent release from frequent fire pressure.

taking the fynbos from a non-equilibrium model of population dynamics to one where a steady state exists. This model states that post-fire fynbos communities are subject to variable recruitment resulting in a shifting mosaic of species where “local extinction of community components and invasion (or recolonization) is a normal and regular process”. Aliens disperse into these communities in the same manner as local species, but do not exhibit local extinctions after fire events due to their quick achievement of reproductive maturity and copious seed production (Richardson and Cowling 1992). Indeed, *A. saligna* produces an average of 10,000 seeds m⁻² of canopy cover (Milton and Hall 1981) and drastically alters native species composition and seed bank stores underneath its canopy (Holmes and Cowling 1997 b). Shade intolerance probably causes fynbos natives to drop out of the system underneath the dense canopies of acacias, with only bird dispersed plants persisting due to constant seed input in the acacia thickets. Most fynbos species, however, have short dispersal distances (Holmes and Cowling 1997 a) causing re-invasion by native elements to be difficult at best. Although some native species persist in the seed bank, large serotinous reseedling shrubs, including proteas, drop out entirely (Holmes and Cowling 1997 b).

In addition to these community level changes, this study has shown that ecosystem function has also been altered. Rates of nutrient turnover have been increased under both invasive alien N₂-fixing species. The shift from low to high N cycling rates can be seen as detrimental for several reasons. First, higher nutrient levels may lead to secondary invasions by weedy grasses and other nitrophilous species (see Section 4.6). Second, edaphic characteristics are thought to be determinants of species composition (Cowling and Holmes 1992, Richards *et al.* 1997 a, b) and speciation patterns (Cowling *et al.* 1992) in the fynbos. Finally, some fynbos species are sensitive to shifts in nutrient levels. For *P. repens*, N:P

imbalances in potting soil decreased growth rates and caused mortality in seedlings (Witkowski 1989 a) while nutrients added in the field decreased growth rates of mature individuals (Lamb and Klaussner 1988). Efforts to restore the old nutrient regime, however, will be fruitless if the cycle of alien recruitment is not stopped. This should incorporate both the eradication of the parent alien plants, the alien seed bank, and the stimulation or addition of the native seed bank. Only by tackling both community and ecosystem changes can the system return to its pre-invasion non-equilibrium state.

The first obvious step in restoration is to clear the alien plants. Current efforts by the Working for Water programme to clear woody alien vegetation involve the cutting of trees with chainsaws or hacksaws and the application of herbicide to the stumps. Lupins will be easier to clear; the lack of native remnant species within old-field sites will allow the use of heavy machinery such as plows and rakes to pull plants and remaining litter. This technique was found successful as primary treatment to clear *Lupinus arboreus* (yellow bush lupin) invasions on Californian coastal sand dunes (Pickart *et al.* 1998 b). Hand pulling and raking will not be difficult in the sandy soils where heavy machinery is precluded due to lack of access or remaining native species. The removal of as much litter and woody biomass as possible from the plots is recommended, since the litter may otherwise increase the normal pulse of N mineralization in fynbos soils after fire (see Section 4.7).

Holmes and Richardson (1999) suggest that planting and harvesting a crop of annuals under long-term acacia invasions in the fynbos might decrease the nitrogen pool in the soil before other restoration efforts are attempted. Within Riverlands Nature Reserve, it will probably not be necessary to plant a crop of annuals because cleared invasions that have not been burned are already colonized by weedy grasses and forbs (see Section 4.6). The harvesting of these "crops" before burning and other restoration efforts may help alleviate the problem of excess nitrogen, but not entirely. Only a small percentage of the organic nitrogen

is mineralized into available forms and taken up by plants each year. Of the gross N mineralization that occurs, plant-microbe competition may account for re-immobilization of the available N, decreasing the net mineralization figure further (Kaye and Hart 1997). Therefore, nitrogen will still exist in the forms of microbial biomass and soil organic matter after one growing season. Indeed, studies have already shown that once elevated, soil N pools do not disappear quickly. Experimental manipulations of ^{15}N labeled *Lupinus angustifolius* litter in sandy soil showed that over 80% of the lupin N remained in an organic form after 6 months and only 9 - 27% was taken up by a mature wheat crop (Russell and Fillery 1999). In experimental removals of *Lupinus arboreus* invasions, where all plants and litter were removed annually, soil organic matter, NO_3^- , and NH_4^+ did not significantly change by the third year (Pickart *et al.* 1998 a). It has been predicted that it will take about 25 years for N pools in soils underneath *L. arboreus* to be reduced by half (Maron and Jefferies 1999). In this study, the more recalcitrant litter and organic matter of acacias will take even longer (see Section 4.4). Slow release of available N after invasions from nitrogen rich organic matter will actually create a more continuous and problematic supply of nutrients than a one time application of fertilizer, the effects of which are no longer noticeable after one year in acid sandplain lowland fynbos soils (Witkowski *et al.* 1990 a).

A different strategy might be to add a mulch with a high C:N to help immobilize the excess nitrogen in the soil. By adding excess amounts of C rich litter to the soil, C:N ratios would increase, creating a N limited system for the microbial community. Although the total N content of the soil would not change, less of the nitrogen would be in plant available inorganic forms because the inorganic N would be re-immobilized into microbial biomass or stable organic N compounds (Cheshire *et al.* 1999). Under these conditions, the high net N mineralization rates under acacia and lupin invasions would decrease and less N would be available for plant uptake.

The use of soil amendments with high C:N ratios have been used previously to lower levels of plant available inorganic N, decrease abundance of alien species and increase abundance of native species (see Wilson and Gerry 1995, Zink and Allen 1998, and Török *et al.* 2000). It should be stressed, however, that although soil amendments immobilize available nitrogen in the short term, they are not changing overall values of total soil N. In the fynbos, the goal should be to immobilize the elevated levels of available N long enough for native species to benefit. The soil C:N should be kept above 30, thereby stimulating immobilization rather than mineralization of nitrogen, until the next fire cycle. This may be able to keep nitrophilous weedy species out of the system in the first post-fire growing season – a time period which is an important determinant for later community structure. It may also give native plants time to produce a sizable seed bank. The elevated total soil N may make the community susceptible to further weed invasion in the long term, so continued management may be needed even if native species are established.

Detailed protocols for restoring fynbos vegetation have been suggested by Holmes and Richardson (1999). Besides offering a different strategy for soil amelioration, only brief restoration suggestions will be made within this manuscript. It is recommended that managers burn once alien vegetation is cleared to remove remaining biomass and help stimulate what is left of the native seed bank to germinate. This will also volatilize biomass, litter, and soil organic N from the upper layers of the soil. Stock (1985) estimated that fire volatilized approximately 24 kg N ha^{-1} , or 20.4% of the aboveground N pool, in acid sandplain lowland fynbos. These figures, however, will be variable based on fire intensity and pre-burn vegetation structure (i.e., how much acacia biomass managers remove and in what form the remaining biomass is stacked.) Because the timing of fire has implications on community regeneration in the fynbos, a cool autumn burn that will then be followed by winter rain may be the most beneficial. In this way, seedling mortality due to lack of

moisture will be lower. Due to the autosuccessional nature of the fynbos, native seed should be added to the plots directly after the fire. Seed should be taken from as close to the restoration area as possible with careful attention directed towards matching phytosociological assemblages based on the edaphic characteristics of particular sites. Mulch should be added to the top layer of the soil without disking or mixing after controlled burning and native seed addition (Zink and Allen 1998). This protocol will act to increase N immobilization, decrease net N mineralization rates, and lower levels of plant available inorganic N without causing further disturbance to the soil. Detailed studies of straw mulch decomposition in soil have shown that near maximum levels of nitrogen immobilization occur within the first month (Cheshire *et al.* 1999). Therefore, it can be expected that the nitrogen will be immobilized quickly by the microbial community soon after it becomes available in early winter (Fig 3.3.2 and 3.3.3).

Changing the nutrient regime of the soil may keep out weedy grasses and forb species, but N₂-fixers will not be limited by decreases in soil N. Post clearing management will need to be in place to make sure that N₂-fixing invasives are not regenerating in the restoration areas, while native species are coming back into the system.

4.9 Conclusions:

Both N₂-fixing invasive alien species in this study, *Acacia saligna* and *Lupinus luteus*, were able to switch and maintain N cycling regimes within the otherwise low nutrient soil of the fynbos. When comparing acacias and lupin to their respective communities (fynbos and grassy old-fields), the N₂-fixing invasives had greater amounts of litter with higher nitrogen concentrations, resulting in more N being returned to the soil from the aboveground biomass. For the acacia stands, these inputs resulted in larger pools of soil total N and organic matter.

Total N and organic matter were not higher in lupin stands due to the quick rate at which the litter decomposed. Rates of N mineralization, however, were increased in the soils of both species; in the form of NH_4^+ and NO_3^- in acacia, and in the form of NO_3^- in lupin.

Differences in the annual and perennial nature of the two invasives are predicted to lead to different patterns in rates of N mineralization through time. Lupin systems are predicted to have a steady state N cycling regime in which annual rates of N mineralization remain constant. Acacia systems are predicted to have increasing pools of soil N and rates of N mineralization through post-fire succession. Comparisons with other studies of N_2 -fixing invasive alien plants suggest that any N_2 -fixer may effect N cycling regimes if the invaded system has low pre-invasion rates of N cycling and enough N rich litter is added by the invasive species through time.

In order to restore fynbos communities, invasive woody plants are now being cleared from large areas of the Cape Floristic Region. Data from this study will help in making predictions about soil N cycling after clear cutting occurs. Although the clear cutting of acacia stands resulted in higher soil temperatures, net N mineralization rates did not increase, probably due to the simultaneous decrease in soil moisture. The current alien plant clearing initiatives in South Africa, therefore, will not result in N mineralization flushes over and above the rates that already exist. It is predicted that fire will decrease N pools and N mineralization rates through N volatilization, but acacia systems will start the next post-fire successional sequence with higher N cycling rates than uninvaded systems. Frequent fires through acacia stands will lead to less pronounced effects of N_2 -fixing invasions because of the constant combustion of organic N pools. In acacia soils, the changes in soil N resulted in greater biomass of a weedy species, forecasting possible feedbacks to community structure after the clearing of aliens during restoration efforts. It is recommended that managers add C rich mulch after fire to areas cleared of N_2 -fixing invasive aliens to reduce the amount of

available nitrogen, aid native species re-establishment, and reduce the competitive advantage of weedy species.

These findings substantiate ideas that species identity can have large effects on ecosystem functioning. Long term invasions of *Acacia saligna* and *Lupinus luteus* in the fynbos were able to switch an ecosystem with slow rates of N cycling into one that operated at faster levels. These changes are predicted to remain in place through positive feedback cycles even after the invasive plants have been cleared.

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7 Appendix 1

Manual Indo-Phenol Blue Determination:

Start with 2 ml of eluate, blanks, or standards and add in order:

1. 2 ml 1.56 M NaOH
2. 1.6 ml 10% (w/v) sodium potassium tartrate solution
3. 0.2 ml 0.16% (w/v) sodium nitroprusside solution made fresh each day
4. 0.4 ml sodium phenate reagent – this was made by dissolving 25 g NaOH in 70 ml water, adding 12.5 g phenol, and making up to 100 ml. The reagent must be made fresh each day.
5. 0.2 ml 3.5% sodium hypochlorite

Mix the reagents and make up to 10 ml with distilled water. Samples, 5 standards, and 2 blanks (5% HCl) were incubated simultaneously in a 40°C waterbath for 20 minutes. The samples were cooled quickly in an ice bath and the absorbance read within 10 minutes at 625 nm. Prepare standards with $(\text{NH}_4)_2\text{SO}_4$ in 5% HCl in the range of 0.6 to 10.8 $\mu\text{g N ml}^{-1}$.

Nitrate Determination:

Copper-Cadmium Reduction:

Start with 3 ml of eluate, blanks, or standards and add:

1. 1 ml 4.8 M NaOH
2. 2 ml 0.4 M NH_4Cl buffer (adjusted to pH 9.6 with concentrated NaOH)
3. approximately 2 g wet weight regenerated Cu/Cd

Shake samples, 5 standards, and 2 blanks (5% HCl) for 10 minutes. Prepare samples with KNO_3 in 5% HCl in the range of 0.6 to 18.0 $\mu\text{g N ml}^{-1}$. Remove 1 ml aliquots for nitrite determination by the Griess-Ilosvay method.

Griess-Ilosvay Nitrite Determination:

Start with 1 ml aliquot from the Cu/Cd reduction and add in order:

1. 1 ml 1% (w/v) sulphanilimide in 5% HCl
2. 1 ml 0.02% (w/v) N-(1-naphthyl)ethylene HCl

Shake reagents and read absorbance after at least 10 minutes at 540 nm (high sensitivity).

Regenerating Copper-Cadmium (from Bates and Heelas 1975):

1. Wash Cu/Cd briefly in 5% HCl
2. Rinse with distilled water
3. Shake Cu/Cd with 0.5% (w/v) $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$ and pour off excess
4. Shake Cu/Cd with a weak acid solution (0.007 N HCl and 0.005 M Na_2EDTA) and pour off several times until the supernatant is no longer black.

Store the Cu/Cd in an airless sealed flask filled with the weak acid solution and wash the Cu/Cd with weak acid solution before and after each use.